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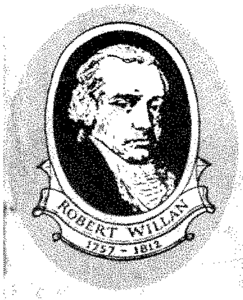

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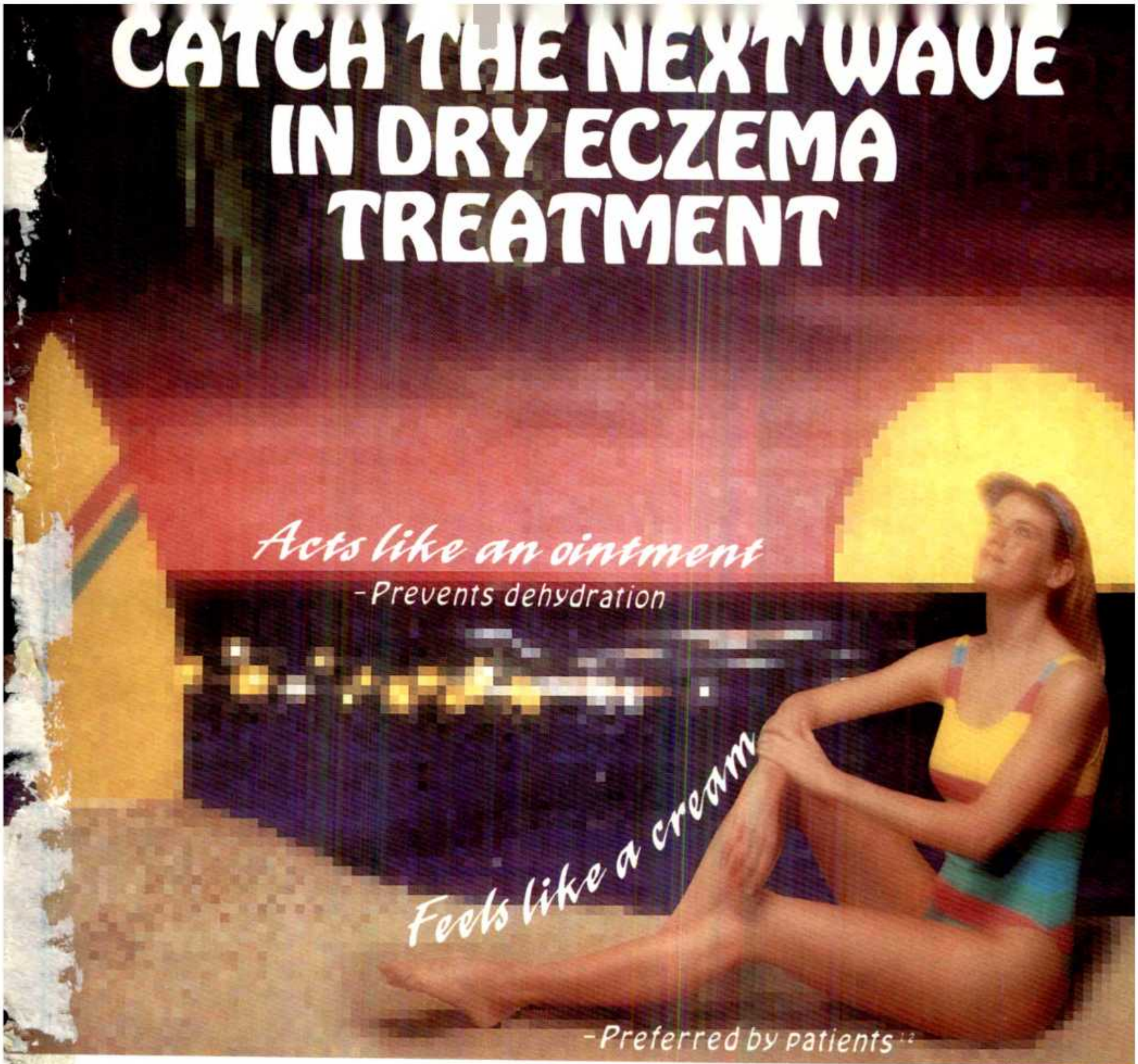

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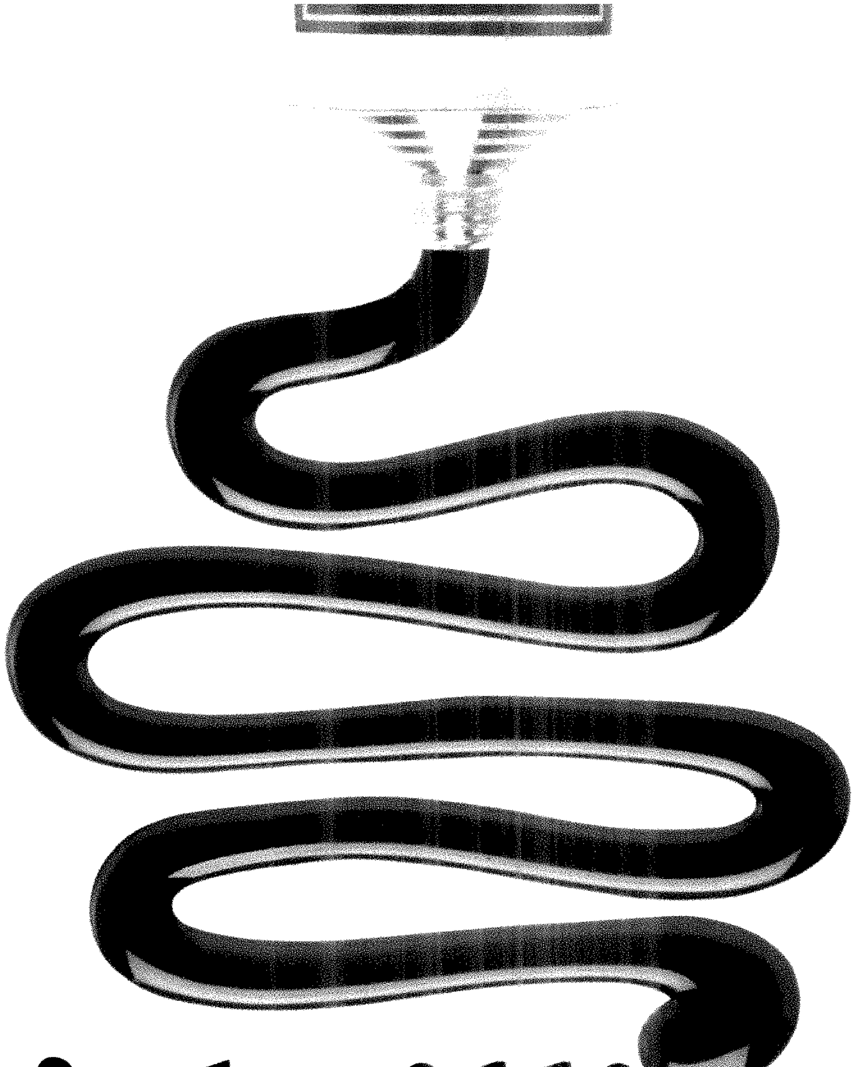

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Type III collagen aminopropeptide and laminin P1 levels in serum of patients with silicosis-associated and idiopathic systemic scleroderma

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SUMMARY

A group of 191 patients with systemic scleroderma and 12 patients with silicosis-associated scleroderma were investigated for connective tissue turnover. The serum levels of type III collagen aminopropeptide (P-III-P), the laminin P1 (Lam P1) fragment and the acid lysosomal β-galactosidase (β-Gal) were determined by specific radioimmunoassays and spectrofluorometry, respectively. Increased levels of type III collagen aminopropeptide strongly correlated with enhanced activity of β-galactosidase. Both parameters correlated with the clinical course in idiopathic systemic scleroderma and in silicosis-associated scleroderma. Serum levels of Lam P1 were also found to be elevated in both groups, although there was no correlation with the severity of the disease. Autoantibodies directed against the DNA topoisomerase Scl-70 and against centromeric proteins were found in a similar range in patients with idiopathic systemic and silicosis-associated scleroderma. These results suggest that P-III-P, Lam P1 and β-Gal are useful serological markers of fibrotic activity and demonstrate similarities between idiopathic systemic scleroderma and scleroderma associated with silica-dust exposure.

Systemic scleroderma (SS) is characterized by progressive fibrosis of the dermis as well as of internal organs.[1-5] Dysregulation of fibroblast metabolism resulting in excessive extracellular matrix production seems to play a major role in the pathogenesis of SS, although the initial events are still unknown. While scleroderma-like lesions have occurred following the occupational exposure to various agents,[6-10] there is a striking association of silica-dust exposure with the development of systemic scleroderma. In a selected group of 86 male SS

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patients diagnosed according to the criteria of the American Rheumatism Association,[11] up to 66 (73%) had been occupationally exposed to silica dust.[12]

Collagen is synthesized in a precursor form as procollagen and after secretion of the molecule into the extracellular space the N- and C-terminal procollagen peptides are cleaved off to then act as feedback inhibitors.[13,14] The serum concentration of the cleaved aminopropeptide of type III collagen (P-III-P) has been shown to reflect the progression of systemic scleroderma.[15-17] Besides the excessive accumulation of connective tissue within the extracellular space, alterations of the vessel wall of capillaries and arterioles have been shown to occur in the early stage of the disease[18-20] which affects the metabolism of basement membrane components such as collagen type IV and laminin.[21] Recently, a radioimmunoassay measuring a pepsin-resistant fragment of laminin (Lam P1) has been established.[22] The serum concentration of Lam P1 is thought to reflect the metabolism of laminin and was found to be elevated in scleroderma patients.[23] As shown in previous studies, the activity of lysosomal β-galactosidase (β-Gal) is also significantly increased in the serum as well as in the skin in scleroderma patients.[24-26]

The aim of the present study was to investigate levels of P-III-P, Lam P1 fragments and β-Gal in order to assess the correlation of these parameters with the clinical course of idiopathic SS and silicosis-associated SS.

METHODS

Patients

One-hundred and ninety-one patients with systemic sclerosis diagnosed according to the American Rheumatism Association criteria[11] were investigated and compared to a group of 12 patients (Table 1) with a history of occupational exposure to silica dust. Ten of the 12 patients with silicosis-associated scleroderma had positive antinuclear antibodies. Four of them had antibodies against centromeric proteins and three against the DNA topoisomerase Scl-70 (Table 2). The diagnosis of silicosis was established by pulmonary changes as seen on the chest X-ray, i.e. nodule formation, calcification, pleural plaques and conglomerate fibrosis. In

TABLE 1. Clinical data of patients with silicosis-associated scleroderma

Patients	Age	Type	Silica exposure time	Organ involvement*						
				R	J	O	L	H	M	K
1 BJ	57	I	6	–	+	–	+	–	+	–
2 BE	71	I	6	+	+	+	+	+	–	–
3 CH	59	I	11	+	+	–	+	+	–	–
4 KM	62	I	3	+	+	+	+	+	–	–
5 KR	57	I	30	+	+	+	+	–	–	–
6 LH	56	III	6	+	+	+	+	–	+	+
7 SchH	58	II	14	+	+	+	+	–	–	–
8 SR	40	I	11	+	+	+	+	–	–	–
9 SpW	58	II	34	+	+	+	+	–	–	–
10 WM	57	III	11	+	+	+	+	–	–	–
11 WH	63	I	3	+	–	+	+	+	–	–
12 ZH	64	II	33	+	+	+	+	+	–	–

* R, Raynaud; J, joints; O, oesophagus; L, lungs; H, heart; M, muscle; K, kidney.

TABLE 2. Autoantibodies in patients with silicosis-associated scleroderma

Patients	Antinuclear antibodies* Titre	Antinuclear antibodies* Fluorescence pattern	Scl-70† antibodies
1 BJ	1:80	Centromeric	–
2 BE	1:1280	Centromeric	–
3 CH	1:100	Nucleolar	–
4 KM	1:80	Centromeric	–
5 KR	—	—	–
6 LH	1:512	Nucleolar	+
7 SchH	—	—	–
8 SR	1:320	Speckled	+
9 SpW	1:10240	Nucleolar	–
10 WM	1:640	Centromeric	–
11 WH	1:5120	Nucleolar	+
12 ZH	1:80	Centromeric	–

* Antinuclear antibodies (ANAs) and their fluorescence pattern were determined by indirect immunofluorescence using Hep 2 cells as substrate (Kallestad, Freiburg, F.R.G.).

† Antibodies against Scl-70 (DNA-topoisomerase I) were determined by double-immunodiffusion using commercial rabbit thymus nuclear extract (Pel-Freeze, Rogers, AK, U.S.A.) as antigen source.

selected cases the diagnosis was confirmed by lung biopsy and by mediastinoscopy. According to their skin involvement, the patients were classified into subsets:[27] type I patients showed involvement of the hands up to the wrist; type II patients displayed additional involvement of areas proximal to the wrist; and in type III patients the fibrosis had started on the trunk and then had spread widely. The reference values for the serological investigations were established from the sera of 149 healthy adults and from 21 patients with silicosis but without evidence of SS. The serum γ-glutamyl transpeptidase, aldolase, cholinesterase, lactate dehydrogenase, glutamate pyruvate transaminase (GPT) and glutamate oxaloacetate transaminase (GOT), as well as the bilirubin and creatinine levels were determined. Patients with increased serum levels indicating liver dysfunction were excluded from the study.

Sera

Blood from patients and controls was allowed to clot for 60 min at room temperature and to retract for 120 min at 4°C. After centrifugation for 10 min at 4°C, serum was aliquoted and stored at −20°C.

Immunoassays

The radioimmunoassay for procollagen III peptide (Fab) was carried out as previously described,[17] with the following modifications. After incubation of the sample with the Fab fragment, immune complexes were precipitated with goat anti-rabbit γ-globulin in the presence of 6% (w/v) PEG 6000. After 20 min at 4 °C, the precipitate was then centrifuged at 3000 **g** for 20 min. The radioimmunoassay for Lam P1 was carried out according to Risteli *et al.*[22] using a commercial test kit (Behringwerke AG, Marburg, F.R.G.). The results were expressed as U/ml and 1 U/ml was defined as the mean concentration of laminin P1 fragment found in normal human sera. The activity of β-galactosidase was determined as previously described.[24] The enzymatic activity is given as nmol of 4-methylumbelliferone released per second under the conditions of the respective assay.

Statistical analysis

Statistical analysis was carried out using the Wilcoxon–Mann–Witney-test.[28]

RESULTS

The levels of Lam P1, P-III-P and β-Gal in the serum of 191 scleroderma patients were determined and compared with those of normal controls. As shown in Table 3, all three parameters were found to be significantly increased in scleroderma patients ($P \leq 0{\cdot}001$). No significant differences compared to controls were detectable in the type I patients with the least severe involvement, but the serum levels in type II patients and, to an even greater extent, in type III patients, greatly exceeded those of the controls. There was a significant correlation between the serum concentration of P-III-P and β-Gal (Fig. 1), but no correlation was observed between Lam P1 and β-Gal or P-III-P. Only P-III-P and β-Gal correlated with the clinical course of the disease with higher levels being seen in the early stages which are characterized by high fibrotic activity and rapid progression of skin and internal organ involvement. These results are in agreement with previous observations,[15,25] confirming the use of all three parameters as laboratory markers for scleroderma.

As shown in Table 4, serum levels of P-III-P, Lam P1 and β-Gal were also significantly elevated ($P \leq 0{\cdot}001$) in patients with silicosis-associated scleroderma, the mean values being similar to those in patients with idiopathic scleroderma. In contrast, in those patients with silicosis without scleroderma, none of the serum levels differed significantly from the normal controls (Table 4). The patients in the silicosis group and those with silicosis-associated scleroderma had a mean duration of exposure to silica dust of approximately 14 years, the range being from 3 to 34 years (Table 1). Clinical impairment in the patients with silicosis-associated

TABLE 3. Serum levels of P-III-P, Lam P1 and β-Gal in patients with idiopathic systemic scleroderma

Patients	P-III-P (ng/ml)	(n)	Lam P1 (U/ml)	(n)	β-Gal (nmol/l per s)	(n)
Scleroderma (total)	74·3 ± 24·6*	(191)	2·2 ± 0·3*	(186)	16·4 ± 4·3*	(126)
Scleroderma (type I)	58·8 ± 20·5	(61)	1·9 ± 0·6	(59)	9·8 ± 4·9	(55)
Scleroderma (type II)	70·9 ± 26·7*	(78)	2·1 ± 0·6*	(75)	14·2 ± 6·6*	(75)
Scleroderma (type III)	96·1 ± 38·1*	(45)	2·4 ± 0·6*	(43)	20·9 ± 6·1*	(45)
Controls	38·4 ± 14·2	(40)	1·4 ± 0·2	(40)	7·6 ± 2·1	(109)

P-III-P, collagen type III aminopropeptide; Lam P1, laminin fragment P1; β-Gal, β-galactosidase; (n), number of patients.

* Significant differences compared to controls ($P \leq 0{\cdot}001$).

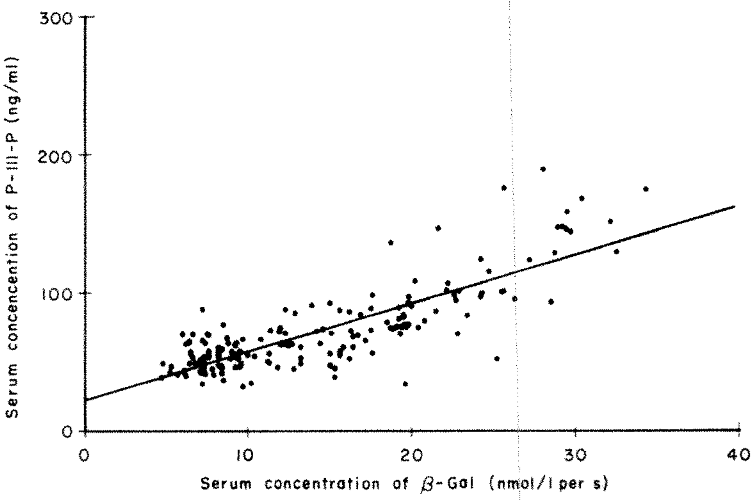

FIGURE 1. Correlation between collagen type III aminopropeptide (P-III-P) and β-galactosidase (β-Gal). Serum levels of P-III-P were determined by radioimmunoassay and are expressed as ng/ml serum. β-Gal was estimated fluorimetrically and is expressed as nmol/l serum per s. $R = 0{\cdot}817$.

TABLE 4. Serum levels of P-III-P, Lam P1 and β-Gal in patients with silicosis-associated scleroderma and patients with silicosis

Patients	P-III-P (ng/ml)	Lam P1 (U/ml)	β-Gal (nmol/l per s)
1 BJ	55·0	2·06	17·4
2 BE	58·2	2·34	16·8
3 CH	62·7	2·10	19·2
4 KM	87·0	2·93	15·6
5 KR	47·8	1·63	18·0
6 LH	168·1	2·84	24·0
7 SchH	145·7	2·71	9·6
8 SR	62·8	1·61	10·8
9 SpW	82·5	2·46	13·5
10 WM	73·4	2·62	30·0
11 WH	64·9	2·71	13·5
12 ZH	74·3	2·51	18·2
× ± SD	81·9 ± 37·1*	2·4 ± 0·4*	17·2 ± 5·6*
Silicosis	50·2 ± 16·3 (*n* = 21)	1·3 ± 0·4 (*n* = 13)	9·1 ± 2·9 (*n* = 21)
Controls (*n* = 40)	38·4 ± 14·2	1·4 ± 0·2	7·2 ± 1·9

β-Gal, β-galactosidase; Lam P1, laminin P1 fragment; P-III-P, aminopropeptide type III collagen.

* Significant at $P \leq 0{\cdot}001$.

scleroderma resulted primarily from involvement of the lung, the gastrointestinal tract and the joints. Seven of the patients were considered to be type I, three patients type II, and two patients type III scleroderma. A rapid deterioration in the clinical status was reflected by highly elevated levels of P-III-P and β-Gal.

DISCUSSION

The primary events in the pathogenesis of SS are still unknown. There are early vascular changes [1-5, 20] and dysregulation of functions of the fibroblasts that are possibly mediated by specific cytokines.[3,29,30] The sporadic occurrence of scleroderma-like changes of the skin in patients suffering from silicosis[31-34] has led to the investigation into whether there is a correlation of silica-dust exposure with SS. Based on previous observations,[12] Haustein *et al.* studied a selected group of 120 male scleroderma patients and found concomitant silicosis in 42% and a positive history of exposure to silica dust in 77% of the patients.[35] It could not be determined whether their disease was mimicking the symptoms of idiopathic scleroderma or whether it was the same disease. The latter possibility implies that SS can be induced by an external agent such as silica dust. The antigen specificity of the antinuclear antibodies (against anti-Scl-70 and anti-centromeric proteins) as well as the type of organ involvement were not distinguishable in patients with silica-associated scleroderma and patients with idiopathic SS.

Analysing additional markers in the serum of SS patients that reflect connective tissue turnover, we found significantly elevated levels of P-III-P, Lam P1 and β-Gal compared to normal controls and a group of silicosis patients without SS. These markers were often elevated in the fibrotic processes[15,36,37] and seemed to reflect the pathological changes associated with and shared by idiopathic systemic and silicosis-associated scleroderma. It has been shown that β-Gal and P-III-P were increased in the serum of scleroderma patients[15,16] and we observed a close correlation of P-III-P and β-Gal with the skin involvement (type I-III) and the severity of the disease in both scleroderma groups. The absence of increased serum levels in the patients with silicosis alone suggests a certain disease specificity. It was previously shown that serum levels of the Lam P1 did not correlate with the severity of SS according to clinically defined subsets or the presence of Raynaud's phenomenon.[23] The analysis of a large group of patients with SS in the present study also failed to demonstrate such a correlation, although both studies revealed an increase of Lam P1 fragment in the SS patients compared to controls.

We can speculate that in the predisposed individual silica dust triggers off changes in the metabolism of connective tissue resulting in a systemic scleroderma-like disease. Consequently, altered basement membrane turnover and fibrotic activity is reflected by increased levels of connective tissue protein fragments (Lam P1 and P-III-P) and β-Gal activity in the serum of affected patients. In this regard, silicosis-associated and idiopathic SS are indistinguishable.

ACKNOWLEDGMENTS

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Ciprofloxacin-induced photosensitivity: *in vitro* and *in vivo* studies

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SUMMARY

Ciprofloxacin is one of the new series of broad-spectrum antibiotic quinolones, chemically related to nalidixic acid and which may, therefore, induce photosensitization of human skin. Three *in vitro* tests for phototoxicity: the destruction of histidine, killing of mouse peritoneal macrophages and inhibition of PHA-stimulated DNA synthesis in human lymphocytes have demonstrated this photosensitizing potential with UVA irradiation at an order of magnitude lower than that for nalidixic acid. The *Candida albicans* test and photohaemolysis were negative. Controlled irradiation monochromator phototesting of 12 subjects, before, during and after taking ciprofloxacin showed subclinical photosensitivity with significantly lowered minimal 24 h erythema doses at 335 ± 30 nm, 365 ± 30 nm and 400 ± 30 nm but not at 305 ± 5 nm or above 400 ± 30 nm.

Drug-induced photosensitization, resulting in a varied pattern of abnormal skin reactions to sunlight[1,2] continues to present problems for both the dermatologist and the pharmaceutical industry.[3-5]

Ciprofloxacin (1-cyclopropyl-6-fluor-1,4-dihydro-4-oxo-7-(1-piperazinyl)-3-quinoline carboxylic acid) (Fig. 1) is one of the new series of broad-spectrum antibiotic quinolones. Adverse effects appear to be uncommon but gastrointestinal, central nervous system and skin reactions have been reported.[6] The skin reactions have included mild photosensitivity in a limited number of subjects.[7-9] Ciprofloxacin is chemically related to nalidixic acid (Fig. 2), a known photosensitizer and one of the drugs associated with the development of a fragile skin and the characteristic blistering of pseudoporphyria as seen in sun-exposed areas.[10-13] We have investigated the phototoxic potential of ciprofloxacin using five model systems for phototoxicity[14] and performed routine phototesting procedures[15,16] in 12 subjects taking the drug.

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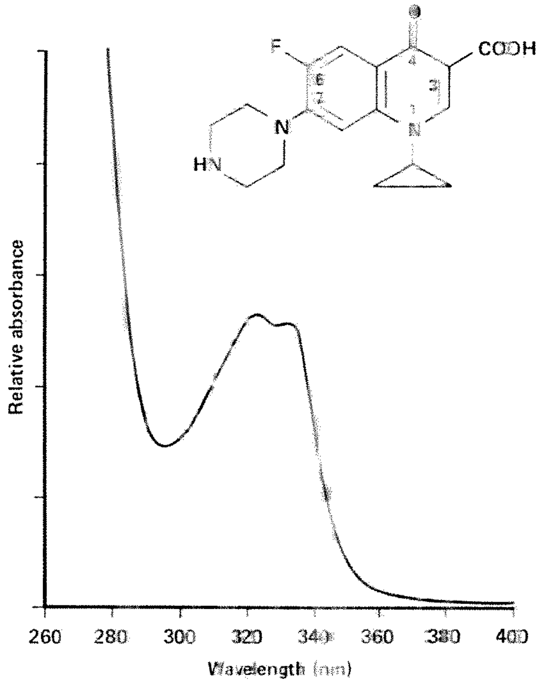

FIGURE 1. Ciprofloxacin absorption spectrum (phosphate buffer), and molecular structure.

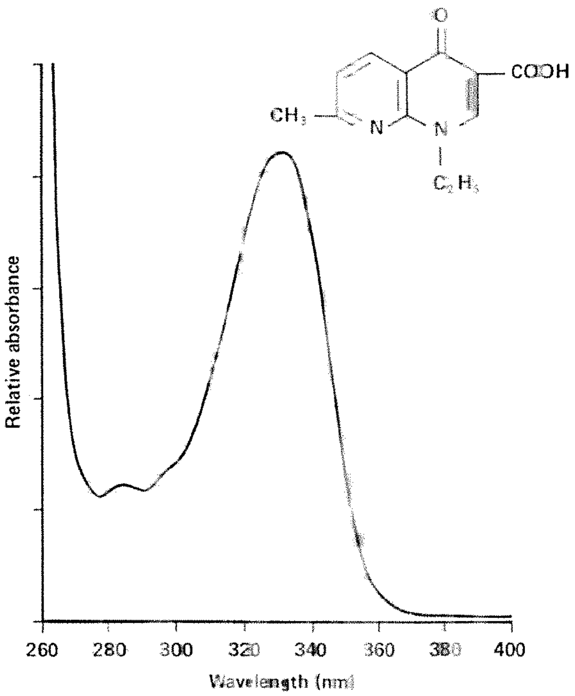

FIGURE 2. Nalidixic acid absorption spectrum (phosphate buffer), and molecular structure.

METHODS

In vitro studies

For the laboratory studies, ciprofloxacin was supplied as a pure compound by Bayer U.K. Ltd, Newbury, Berks, U.K. and prepared as a 1 mg/ml (approximately 2·5 mM) stock solution in Michaelis barbitone-buffered saline at pH 7·2. Nalidixic acid (Sterling–Winthrop Group Limited) was prepared as an approximately 21·5 mM stock solution by suspending 10 mg in 0·5 ml distilled water, adding 0·25 ml 1 N NaOH, and making up to 2 ml with barbitone-buffered saline. Dilutions for working solutions were made in Michaelis-buffered saline, phosphate-buffered saline or cell-culture medium depending on the *in vitro* test to be used.

Five *in vitro* tests[14,17,18] were used to determine the phototoxic potential of ciprofloxacin.

Candida albicans. The potential photosensitizer, either as solid material or as a solution applied to filter paper discs and dried, was placed on a newly seeded culture of *Candida albicans* grown on Sabouraud's agar. Duplicate plates were set up for each test, one being exposed to a low irradiance of UVA (0.2 mW/cm^2) and the other kept as a dark control. After 48 h at room temperature the phototoxicity was assessed by the inhibition of yeast growth around the drug in the irradiated culture while growth was complete in the dark control. A positive control was provided by 8-methoxypsoralen.

Photohaemolysis. Phototoxicity was assessed by the release of haemoglobin, determined as cyanmethaemoglobin, from a monolayer prepared from a 1 : 1000 dilute suspension of red blood cells in barbitone-buffered saline at pH 7·4 after mixing with potential photosensitizer, irradiation (UVA 14 J/cm^2) and standing for 1 or 24 h.

Photosensitized inhibition of DNA synthesis in human lymphocytes. This is an adaptation of the standard immunocytochemical method for determining lymphocyte responses to the mitogen, phytohaemagglutinin (PHA). Phototoxicity was assessed by the inhibition of PHA-induced DNA synthesis measured by [^{3}H]-thymidine uptake in microcultures of lymphocytes exposed to photosensitizer and irradiation.

Photosensitized destruction of histidine. Phototoxicity was indicated by the loss of the 'Pauly' colorimetric reaction for the histidine imadazole ring after irradiation of the amino acid in buffer and drug solution.

Photosensitized killing of mouse peritoneal macrophages. Mouse peritoneal macrophages, which were fully differentiated non-dividing cells, were grown in short-term culture on rectangular cover slips in Leighton culture tubes. After the addition of the supposed photosensitizer, one half of the culture was exposed to UVA and the other half kept as the dark control. Twenty-four hours after exposure the cultures were washed, fixed and stained and phototoxicity assessed by both gross and microscopic observation. Unequivocal phototoxicity was determined as the absence of intact cells in the exposed half of the culture.

Broad band UVA (315–400 nm) was obtained with an array of Sylvania (PUVA) fluorescent tubes (irradiance at exposed surface, determined with Waldmann PUVA meter: 2 mW/cm^2; for *Candida* test: 0·2 mW/cm^2). Broad band UVB was provided by an array of Philips TL20/12 fluorescent tubes (irradiance at exposed surface, determined with calibrated Rank–Hilger thermopile FT17: 1 mW/cm^2). For wavelength-dependency studies a compact, irradiation

TABLE 1. Details of patients treated with ciprofloxacin

Subject	Age	Sex	Skin type	Ciprofloxacin dose duration	Other drugs
1	64	M	I	750 mg b.d. 12 days	Senokot, temazepam, ferrous sulphate
2	64	M	II	750 mg b.d. 7 days	—
3	60	M	III	750 mg b.d. 12 days	Ranitidine, atrovent
4	25	M	I	200 mg i.v. b.d. 2 wks	Salbutamol, paracetamol
5	31	F	IV	500 mg q.d.s. 7 days	—
6	31	M	IV	500 mg q.d.s. 7 days	—
7	56	M	IV	750 mg b.d. 11 days	Prednisolone, ranitidine, mucogel, atrovent, salbutamol
8	49	F	III	500 mg q.d.s. 10 days	—
9	75	F	III	750 mg 7 days	—
10	87	F	I	750 mg 7 days	—
11	84	F	I	250 mg b.d. 7 days	—
12	84	F	IV	750 mg b.d. 10 days	—

NB. No preceding photosensitivity, no photosensitivity while taking ciprofloxacin and no other adverse events.

monochromator (Hilger and Watts D330, symmetrical Czerny–Turner, single plane diffraction grating, 1200 lines/mm, reciprocal dispersion, 2·6 nm/mm with a 450 W xenon arc as source) was used with 1/2 max bandwith, 7·8 nm.[19]

In vivo studies

For the clinical studies, ciprofloxacin was supplied as 250 mg tablets (Bayer U.K. Limited). Twelve subjects (six males, age range 25–64 years, mean 50 years, and six females, age range 31–87 years, mean 68 years) undergoing treatment with ciprofloxacin were studied (Table 1). The doses of ciprofloxacin, taken for a minimum of 7 days, ranged from 250 mg b.d. to 500 mg q.d.s. orally and one patient was treated with 200 mg b.d. intravenously. Exclusion criteria for this study were pregnancy, previous clinical photosensitivity, concomitant photoactive medication and impaired hepatic or renal function. An open study was designed in which standard phototesting procedures with an irradiation monochromator[15-18] were used to determine the photosensitivity of the subjects before taking ciprofloxacin where possible, and again after at least 1 week of treatment, with repeat phototesting at 2-weekly intervals for 4 weeks if a persistent abnormality was detected. For subjects 1–8, a standard range of wavebands, 305 ± 5 nm in the UVB, and 335 ± 30 nm and 365 ± 30 nm in the UVA region was used and then, for subjects 9–12, the phototesting programme was extended to include 400, 430 and 460 ± 30 nm. The skin reactions to an initial large step, graded series of exposures were monitored to examine for abnormal immediate and delayed erythema and to provide an estimate of the minimal erythema dose (MED), at 1, 2, 5, 30 and 60 min and at 4, 24, and when possible 48 h after exposure. A more precise MED, defining the baseline delayed-erythema response for each waveband, was determined by repeating the phototesting on the second day using a small-step series (20%) of exposure doses below and including that estimated as the MED from day one. In five subjects baseline assessment of photosensitivity was possible before treatment with ciprofloxacin and phototesting was repeated at least 7 days after taking the drug at the prescribed dose (Table 1). In all the other subjects, the first phototesting was carried out while taking the drug. A comparison of phototesting results on and off the drug (either pre- or post-treatment)

TABLE 2. Normal subject phototesting data Photobiology Unit, Ninewells Hospital

	Monochromator	
Wavelength (nm)	Range for 70% of normals (mJ/cm^2)	Lowest normal (mJ/cm^2)
305 (± 5)	47–82	27
335 (± 30)	3300–6800	180
365 (± 30)	12,000–22,000	8200
400 (± 30)	47,000– > 47,000	47,000
430 (± 30)	> 82,000	> 82,000
460 (± 30)	> 82,000	> 82,000

was assessed for a significant change in MED or an abnormal response as defined by the Photobiology Unit data bank (Table 2). Repeat phototesting was done 2 and 4 weeks after stopping the drug.

During therapy with ciprofloxacin, each subject was assessed for the presence or absence of photosensitivity. The clinical examination concentrated on the classical phototoxic features such as erythema at the exposed site, blistering, milia, pigmentation and photo-onycholysis. Initially, adverse events were enquired about without any leading questions but later specific enquiries were made as regarding any alteration in photosensitivity.

A statistical analysis of the phototesting results on and off ciprofloxacin was made using Wilcoxon's signed rank test. Blood was taken from each subject for routine porphyrin (plasma fluorescence scan) and lupus erythematosus (ANF, anti-Ro and anti-La antibody) screening.

RESULTS

In vitro studies

For ciprofloxacin, results obtained with *Candida* (solid drug, quantified as 2 and 20 μg/6-mm diameter filter paper disc or as saturated solution applied to filter paper disc) were negative. Nalidixic acid at 2 μg/disc also gave negative results but a minimal phototoxicity was demonstrated with 20 μg/disc producing a zone of growth inhibition of diameter 8·79 mm, SD 2·6 mm ($n = 14$) compared to the 21·2 mm, SD 1·64 ($n = 5$) obtained with 2 μg per disc of the positive control 8-methoxypsoralen (8-MOP).

Photohaemolysis with ciprofloxacin (up to 500 μg/ml and 14 J/cm^2 UVA) was negative whether the cyanmethaemoglobin determination was done on supernatant taken 1 or 24 h after the exposure. Nalidixic acid produced evidence of photohaemolysis, but only with high concentrations of drug of > 400 μg/ml and exposure doses of UVA of 14 J/cm^2 when the determination was done 1 h post-irradiation. However, when the assay was done 24 h after the exposure, almost 100% photohaemolysis was obtained with concentrations of nalidixic acid of 50 μg/ml and over, and 14 J/cm^2 of UVA. UVB-induced photohaemolysis was negative up to 400 μg/ml while the positive control of benoxaprofen produced 100% photohaemolysis at 1·0 μg/ml.

Despite the large degree of variation in the responses of lymphocytes in growth medium alone to UVA, a drug concentration and UVA dose-dependent inhibition of DNA synthesis in PHA

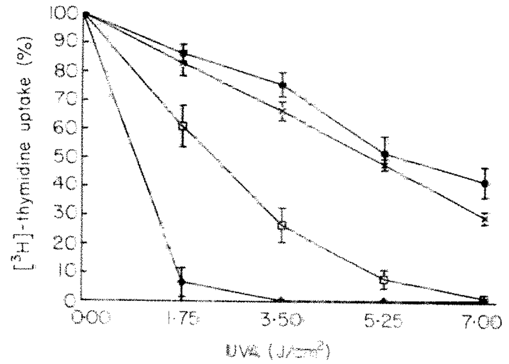

FIGURE 3. Nalidixic acid photosensitized inhibition of DNA synthesis in PHA-stimulated lymphocytes. Bar = standard error. Concentration µg/ml (number of assays per point): ●, 0 (10); ×, 0·5 (8); □, 5·0 (12); ◆, 50 (12).

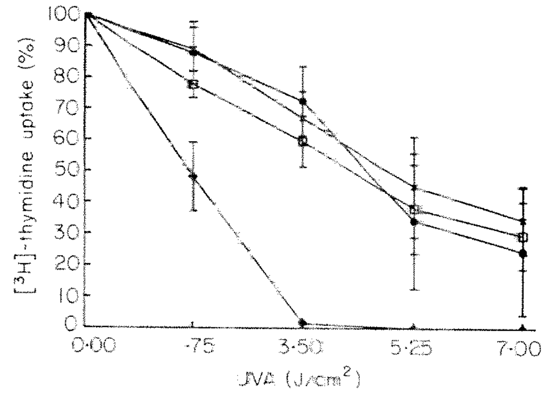

FIGURE 4. Ciprofloxacin photosensitized inhibition of DNA synthesis in PHA-stimulated lymphocytes. Bar = standard error. Concentration µg/ml (number of assays per point): ●, 0 (4); ×, 0·5 (6–12); □, 5·0 (8–12); ◆, 50 (12).

stimulated lymphocytes was demonstrated for both nalidixic acid and ciprofloxacin (Figs 3 and 4). Using a two-sided '*t*'-test to compare the results with those for growth medium controls, phototoxicity with nalidixic acid at a concentration of 0·5 µg/ml approached statistical significance only with a UVA exposure dose of 7 J/cm². With 5 µg/ml, there was significant phototoxicity at 1·75 J/cm² and higher exposures. With 50 µg/ml, and 1·75 J/cm² UVA, phototoxicity was significant at $P < 0{\cdot}01$. Ciprofloxacin at concentrations of 0·5 and 5·0 µg/ml was not phototoxic in this model but at 50 µg/ml, 1·75 J/cm² UVA and above, produced a statistically significant effect ($P < 0{\cdot}0001$). Phototoxicity with 8-MOP positive controls reached statistical significance with 5 ng/ml ($P = 0{\cdot}004$) and 1·75 J/cm².

Neither ciprofloxacin nor nalidixic acid at concentrations of 50 µg/ml pre-irradiated in phosphate-buffered saline at pH 7·2 and then added to lymphocytes before stimulation with PHA had an effect in this model.

Using a standard UVA exposure dose of 14 J/cm² both nalidixic acid and ciprofloxacin produced a concentration-dependent photosensitized destruction of histidine (Table 3). A best

TABLE 3. Photosensitized destruction of histidine. Percentage histidine remaining after 14 J/cm² UVA, mean ± SD (*n*)

Concentration (μg/ml)	Nalidixic acid	Ciprofloxacin
0·5	99·75 ± 0·5 (3)	—
5·0	68·5 ± 7·5 (4)	100·0 ± 0·0 (4)
50·0	12·3 ± 6·6 (4)	65·4 ± 6·0 (7)
100·0	—	48·75 ± 9·1 (4)
200·0	—	30·0 ± 4·2 (4)

TABLE 4. Wavelength dependence for macrophage photosensitization[19] with 50 μg/ml ciprofloxacin and nalidixic acid

	Minimal lethal dose J/cm²								
Wavelength (nm)	315	320	325	330	335	340	345	350	355
Ciprofloxacin	0·51	0·96	**0·42**	**0·66**	**0·68**	1·16	2·36	> 3·0	> 5·0
Nalidixic acid	1·12	**0·79**	**0·77**	**0·81**	**0·83**	1·17	2·12	> 4·0	> 4·0

Each data point was obtained from at least three experiments. For each waveband used, the all or none type of response produced the same killing dose in each experiment. Most active wavelengths are indicated by bold type.

fit straight line obtained from a plot of histidine remaining against the log of the drug concentration allowed the determination of a 50% effective concentration for nalidixic acid of 8·5 μg/ml and for ciprofloxacin of 94·5 μg/ml.

Using ciprofloxacin at 25 μg/ml and 10·5 J/cm² UVA or 50 μg/ml and 3·5 J/cm² mouse macrophages were damaged and cells present in the irradiated field had increased cytoplasmic vacuolation or a shrunken, deep staining cytoplasm. No cells were present in the irradiated field with 50 μg/ml ciprofloxacin and 7 J/cm² UVA and above, and with 100 μg/ml and 3·5 J/cm² UVA and above. An action spectrum for this killing effect showed a peak around 325–335 nm (Table 4).

With nalidixic acid, cell damage was obtained with 10 μg/ml and 3·5 J/cm² UVA while cell death was produced with higher UVA doses and at all exposure doses with 50 μg/ml and above. As with ciprofloxacin, the wavelength dependency for this phototoxicity was in the 320–335 nm region (Table 4).

In vivo studies

All 12 subjects studied were Caucasian with the range of skin types seen in the local population (Table 1). Clinical assessment failed to reveal evidence, from history or clinical examination, of an acute photosensitivity episode or phototoxic sequelae during the time of the study (Table 1) nor were other adverse effects reported. Porphyrin plasma scan and lupus erythematosus (ANF, anti-Ro, anti-La) studies failed to show abnormalities before or after taking the drug in all subjects tested.

TABLE 5. Minimal erythema dose (MED) determinations for the 305, 335, 365 and 400 nm wavebands before, during and at 2 weeks after taking ciprofloxacin

Waveband	Subject no. 1	2	3	4	5	6	7	8	9	10	11	12
305 ± 5 nm mJ/cm²												
Pre	47	NT	NT	NT	33	33	NT	NT	NT	NT	47	47
On	39	47	39	> 47	> 47	33	39	47	56	39	> 47	47
2 weeks post	NT	47	47	> 47	NT	33	> 47	47	56	47	NT	NT
Lowest normal population MED: 27 mJ/cm²												
335 ± 30 nm J/cm²												
Pre	3·9	NT	NT	NT	> 3·9	> 3·0	NT	NT	NT	NT	2·2	4·7
On	2·7	1·8	1·5*	3·3	2·7	1·2*	2·2	2·2	2·2	1·2*	0·82*	1·2*
2 weeks post	NT	3·3	3·9	3·9	NT	3·3	> 4·7	4·7	> 4·7	> 4·7	NT	NT
Lowest normal population MED: 1·8 J/cm²												
365 ± 30 nm J/cm²												
Pre	12	NT	NT	NT	12	> 12	NT	NT	NT	NT	12	18
On	> 12	8·2	10	> 12	10	5·6*	8·2	8·2	5·6*	5·6*	4·7*	5·6*
2 weeks post	NT	12	> 12	> 12	NT	> 12	> 12	15	18	> 18	NT	NT
Lowest normal population MED: 8·2 J/cm²												
400 ± 30 nm J/cm²												
Pre	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	> 47	> 47
On	NT	NT	NT	NT	NT	NT	NT	NT	22*	33*	47	47
2 weeks post	NT	NT	NT	NT	NT	NT	NT	NT	47	> 47	NT	NT
Lowest normal population MED: 47 J/cm²												

NT = not tested.

* Abnormal phototest response.

TABLE 6. MED for threshold delayed erythema for UVB and UVA wavebands with and without ciprofloxacin

	Mean for 12 subjects (SD)		
	305 ± 5 nm mJ/cm²	335 ± 30 nm J/cm²	365 ± 30 nm J/cm²
MED without ciprofloxacin	45·4 (6·4)	4·04 (0·75)	13·75 (2·8)
MED with ciprofloxacin	43·9 (6·2)	1·92 (0·75)	7·97 (2·4)
'*P*' value (Wilcoxon)	NS	<0·001	<0·01

NS, not significant.

Baseline MEDs in those subjects tested prior to taking ciprofloxacin were within normal limits at all wavelengths tested (Table 5) as defined by our normal population MED database.

While taking ciprofloxacin, six subjects (50%) demonstrated abnormal phototest responses (Table 5). These were low MEDs for delayed erythema as determined by readings at 24 h, confined to the wavebands from 335 ± 30 nm to 400 ± 30 nm. In all the subjects there was no significant change in MED at 305 ± 5 nm (Tables 5 and 6; Fig. 5) but a reduction in MED at 335 ± 30 nm ($P < 0{\cdot}001$) was evident while on the drug (Tables 5 and 6; Fig. 5) and with 365 ± 30 nm, this result ($P < 0{\cdot}01$) was obtained in 10/12 subjects (Tables 5 and 6; Fig. 5). In the four subjects tested at 400 ± 30 nm, there was a similar reduction in MED while taking the drug (Table 5) but this change could not be detected at 430 ± 30 nm and 460 ± 30 nm in the two subjects who were also tested at these visible wavelengths.

Forty-eight-hour readings, when undertaken, revealed only fading erythema, suggesting that abnormal erythema, when present, was maximal around 24 h after testing. In no instance was a dermatitis response observed. No abnormal immediate erythema was observed, nor was there

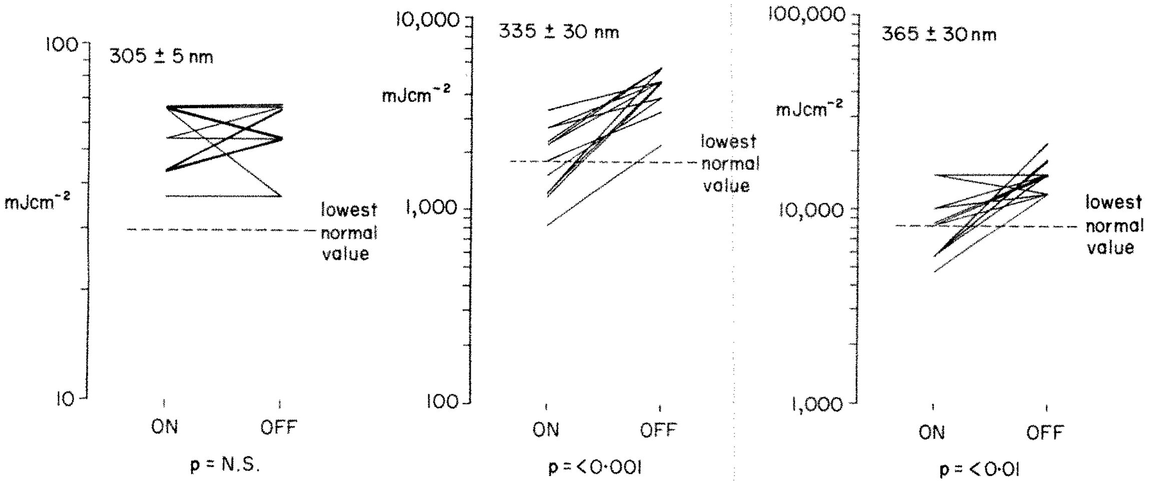

FIGURE 5. MEDs at 305 ± 5 nm, 335 ± 30 nm and 365 ± 30 nm for 12 subjects while taking (ON) and not taking (OFF) ciprofloxacin.

evidence of an abnormal sensation, urticaria or blistering. All phototest reactions resolved leaving clinically normal skin.

In all subjects, MEDs determined 2 weeks after stopping the drug were within normal limits and, for the UVA wavelengths, were at higher levels than those obtained while ciprofloxacin was being taken. There was no evidence, therefore, of a persistent photosensitivity with this drug.

DISCUSSION

We have shown that one of the new quinolone carboxylic acid broad spectrum antibiotics, ciprofloxacin, has a phototoxic potential *in vitro* and that the most effective wavelength for this effect is around 325 nm. Other compounds of this type have also been reported to have varying degrees of phototoxic potential *in vitro*.[20] For ciprofloxacin, the pattern of this *in vitro* phototoxicity is similar to that for nalidixic acid but in terms of drug concentration and UVA dose requirement, ciprofloxacin appears to have a lower phototoxic potential. It was possible to obtain photohaemolysis with nalidixic acid but not with ciprofloxacin. This suggests that while membrane-dependent reactions may play a major role in nalidixic acid phototoxicity, ciprofloxacin-induced phototoxicity may be mediated more through an intracellular target. Normal plasma levels of ciprofloxacin after the dose regimen given to our subjects are in the range of 1–5 μg/ml. Although no direct relationship between drug plasma levels and concentrations required for *in vitro* phototoxicity has been established, some build up of ciprofloxacin in the skin would have to occur to reach the 50 μg/ml which produced unequivocal phototoxicity in our tests. The UVA levels required however, are easily attained since the exposure dose of about 3·5 J/cm^2, is equivalent to approximately 12 min exposure to midday, midsummer sunlight in Dundee as determined with a Waldmann PUVA meter.

Ciprofloxacin is photodegraded by the exposure doses and wavelength range of UVR which produce this phototoxicity,[21] but, as with nalidixic acid, the photoproducts formed are not toxic for responses of human lymphocytes to PHA. It should be emphasized that the two simplest and most commonly used models for phototoxicity, photohaemolysis and *Candida albicans*, were negative for ciprofloxacin. This illustrates the importance of using a battery of tests *in vitro* unless one complex, cellular model has been validated.

This investigation has shown that the phototoxic potential of ciprofloxacin demonstrated *in vitro* may be realized in a group of subjects taking therapeutic doses of the drug as subclinical photosensitivity, which is only revealed by phototesting. However, it is clear that ciprofloxacin-induced phototoxicity is manifest in human skin by a lowered MED to UVA, particularly around 335 nm, which fits the wavelength dependence for *in vitro* phototoxicity, but also at 365 nm and the wavelength dependency appears to extend into the visible light at 400 nm. Higher levels of the drug in the skin, brought about by retention or other abnormal pharmacology, along with increased environmental UVA levels, may result in clinical presentation of this abnormal photosensitivity. The wavelengths involved indicate that it is possible that sunlight transmitted through window glass and/or thin clothing will produce this photosensitivity response. The degree of reaction obtained suggests that ciprofloxacin is a mild photosensitizer when compared with known, potent systemic photosensitizing agents such as 8-methoxypsoralen, amiodarone, chlorpromazine and benoxaprofen. At no time has the unpleasant sensation reported to accompany the early stages of cutaneous photosensitivity obtained with the last three drugs been a feature with ciprofloxacin.

As with all photoactive agents, the mechanism of action is important in that photoallergy or drug-induced lupus erythematosus may produce significant and persistent clinical problems.

Neither of these two mechanisms appears to play a role with ciprofloxacin. The results of this study point towards a phototoxic mechanism as the cause of abnormal photosensitivity. The phototest morphology is of an asymptomatic erythema and not dermatitis. A change in photosensitivity was observed in all the subjects taking the drug and abnormal responses were obtained in a significant number (50%). The abnormal photosensitivity was reversed within 2 weeks of stopping the drug. Ciprofloxacin is phototoxic *in vitro*, but we were unable to find evidence for any other mechanism of action.

Since a phototoxic mechanism is involved, an abnormal skin reaction will be produced in any patient who has taken enough of the drug and is then exposed to sufficient of the appropriate radiation (in this case UVA and perhaps visible wavelengths of light). It may predicted that in any patient who develops photosensitivity, the effect will be reversible. Improvement may be obtained by reducing the drug dosage or, if necessary, by stopping it altogether which, in this study, resulted in a return of the phototest responses to normal limits within 2 weeks. Any patient who develops a photosensitivity reaction while taking ciprofloxacin should be able to prevent recurrence by the use of an appropriate screening agent designed to provide protection against the UVA and also visible wavelengths. A reflectant barrier containing titanium dioxide or zinc oxide as well as UVA absorbing chemicals should be used but an anti-sunburn, UVB sunscreen will be useless. Further clinical experience and animal experiments should reveal whether the severe cutaneous 'pseudoporphyria' lesions associated with nalidixic acid phototoxicity[9,10,22] might also be produced in subjects taking ciprofloxacin and the other related quinolones. In addition, the importance of the photoproducts of these compounds, not only in terms of reactions induced at the cutaneous site of photodegradation, but also with a view to systemic and, in particular, hepatic toxicity[23,24] require investigation.

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An immunoradiometric assay for von Willebrand factor antigen to assess angiogenesis

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SUMMARY

A method of determining the degree of vascularization of tissues would be of great value in dermatological research such as the investigation of neoangiogenesis in wounds and in the study of the vascular abnormality in psoriasis. We describe an adaptation of an immunoradiometric assay (IRMA) for von Willebrand factor antigen for this purpose. The results show that in endothelial cells in culture there was a good relationship between the number of cells and von Willebrand factor antigen measured by the IRMA. The relationships between the numbers of endothelial cells in biopsies of normal and healing wound skin as estimated by the histometric technique and the IRMA are less good ($r = 0{\cdot}45$ and $0{\cdot}32$, respectively) but suggest that there is a positive and proportionate variation. There is a much stronger relationship ($r = 0{\cdot}79$) between the biochemical and histometric methods in psoriatic skin.

The revascularization of wounds is believed to be a major rate-determining factor in the healing process. For this reason, efforts have been made to improve the rate of neoangiogenesis and to enhance the blood supply to non-healing wounds. The capillary in the papillary dermis in psoriasis appears abnormal but it is not known whether the abnormality persists after treatment or is present in the apparently unaffected skin. It has been claimed that some agents such as topical retinoic acid influence vascularization but the evidence presented is mostly indirect. Unfortunately, current methods to measure angiogenesis are technically laborious if they require histometric evaluation of tissue, or of questionable validity for man if they utilize a non-human model such as the chick chorioallantoic-membrane method.[1] The studies reported here have used the specific localization of von Willebrand factor antigen (vWFAg) to endothelial cells and platelets as the basis of a technique for the measurement of the degree of revascularization.

METHODS

Study design and subjects

The *in vivo* study was divided into two parts, both of which received ethical approval from the

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Joint Ethics Committee of the South Glamorgan Area Health Authority/University of Wales College of Medicine. In the first, six normal volunteer subjects who had given their signed, informed consent had 3 mm punch biopsies taken from the lateral aspect of the upper thigh. Three days later the healing areas were excised using a 6-mm diameter trephine. Both types of sample were divided in two for immunoradiometric analysis of the vWFAg and for the histometric evaluation of the number of endothelial cells.

In the second part of the study, seven patients with typical untreated plaque type psoriasis, who had given their signed, informed consent, were recruited. A 4-mm diameter biopsy was taken from the centre of a psoriatic plaque and another from an uninvolved site on the same body area. Each biopsy was divided into two and the segments treated as previously. Details of the patients are given in Table 1.

In addition, cultured human umbilical vascular endothelial cells were studied to further characterize the relationship between endothelial cell number and their content of vWFAg.

Quantification of vascular endothelium in skin

Formalin-fixed, wax-embedded skin biopsies were sectioned at a nominal thickness of 5 μm using a Bright's microtome. Five slides were prepared from each sample at 100 μm intervals and stained using the periodic acid Schiff reaction followed by haematoxylin. Quantitative analysis was performed using stereological point counting.[2] An eyepiece graticule inscribed with a 10 × 10 regular lattice of small crosses was used and, at × 40 objective magnification, the number of crosses within vessels (V) was counted and within any epidermal, sebaceous gland, hair follicle or other non-dermal tissue (N). Pilot studies have shown that 30 fields (six per section) gave a representative estimate of blood vessel relative volume (Vvv.d), calculated by the formula:

$$\mathrm{Vvv.d} = \frac{V}{(100 \times 30) - N}.$$

Endothelial volume was then determined by observing 25 randomly selected vessels with a × 100 oil-immersion objective lens. The number of points within the endothelium (E) and

TABLE 1. Patient details for comparison of endothelial volume and von Willebrand factor antigen in involved and uninvolved psoriatic skin

No.	Patient age	Sex	Site of biopsy Involved	 Uninvolved
1	69	F	Forearm	Forearm
2	53	F	Leg	Forearm
3	19	F	Leg	Forearm
4	35	F	Forearm	Forearm
5	18	M	Abdomen	Forearm
6	20	F	Hand	Forearm
7	21	F	Forearm	Forearm

lumen (L) was counted. The volume fraction of endothelium in dermis (Vve.d) was then calculated from:

$$\mathrm{Vve.d} = \mathrm{Vvv.d} \times \frac{(\mathrm{E})}{\mathrm{E}+\mathrm{L}}.$$

Preparation of skin homogenates and immunoradiometric assay

The skin biopsy samples were washed extensively in 0·05 mol/l phosphate-buffered saline at pH 7·1 and homogenized in 0·2 ml 0·005 mol/l barbital buffer pH 7·9 containing 1% (v/v) Triton X100 using a mechanical teflon-glass homogenizer (Burkard Scientific Ltd., Rickmansworth Herts, U.K.) The product was frozen and thawed three times. The amount of protein in the homogenate was estimated using the method of Lowry *et al.*[3]

Monoclonal antibodies[4] and polyclonal antiserum[5] to vWFAg were raised as previously described. Rabbit antibody to human albumin together with peroxidase-conjugated antibodies were purchased from Dako (UK) Ltd., High Wycombe, Bucks, U.K.

A two-site immunoradiometric assay for vWFAg was developed using the principles established by Peake and Bloom[6] and detailed by Giddings.[7] Polystyrene tubes (12 × 7 mm, Sarstedt Ltd., Leicester, U.K.) were coated internally by incubation overnight at 4°C with 0·5 ml rabbit antibody to vWFAg diluted 1:2000 in 0·05 mol/l bicarbonate buffer, pH 9·6. The remaining reactive sites were blocked by incubation with buffer containing 1% (w/v) bovine serum albumin. The tubes were then aspirated and washed sequentially with 4 ml 0·05 mol/l barbital buffer, pH 7·9 and 4 ml distilled water. 0·2 ml of dilutions of the tissue homogenates were added to the washed, coated tubes and each tube was stoppered before incubation overnight at 37°C. The tubes were again washed with 4 ml 0·05 mol/l barbital buffer, pH 7·9 containing 0·1% (w/v) bovine serum albumin. A 0·2 ml of a dilution of radiolabelled antibody to vWFAg giving approximately 10,000 counts per minute was added, and the tubes were restoppered and incubated overnight at 37°C and for a further 24 h at 4°C. After incubation the tubes were washed three times with distilled water and counted in a Rackgamma II (LKB Instruments Ltd., S. Croydon, Surrey, U.K.). The bound radioactivity expressed in counts per minute, was calculated after the subtraction of the background count and was shown to be directly proportional to the concentration of vWFAg in the tissue samples compared with a reference curve prepared from dilutions of pooled normal human plasma assayed in each experiment. This in-house, standard plasma was calibrated against the International Reference Preparation, 87/718, supplied by the National Institute for Biological Standards, London. The precise weight of vWFAg in this reference material is not known and values were recorded in International Units (iu.). It is not, therefore, totally appropriate to assess antigen recovery in nanogram amounts. Nevertheless, earlier studies have indicated that normal human plasma contains approximately 10 mg vWFAg per l[8] and on this basis the present IRMA was estimated to be sensitive to approximately 0·1 ng antigen.

Tissue homogenates were also examined for contamination with blood by a conventional ELISA method for human albumin. Microtitre plates (96-well) were coated with unlabelled polyclonal antiserum to human albumin and bound antigen was detected using a second antibody conjugated with peroxidase together with 1,2 ortho-phenylenediamine dihydrochloride (OPD) as an enzyme substrate.

Culture of endothelial cells

Human umbilical cords were obtained at delivery from the Department of Obstetrics of the University Hospital of Wales. Undamaged lengths of cord were selected, sealed in plastic envelopes and processed within 3 h of collection. Endothelial cells were harvested by intraluminal collagenase digestion and cultured in medium 199 (Gibco) supplemented with 20% fetal calf serum as previously described.[9] Briefly, cells were recovered from the umbilical veins after treatment with 0·1% (w/v) collagenase for 10 min at 37°C. Cells were cultured in a humidified atmosphere of 5% CO_2/95% air at approximately 2×10^5 per ml of medium. Penicillin, 200 units per ml, and streptomycin, 200 μg per ml, were added as antibiotics, and amphotericin B, 5 mg per ml, as fungicide, and the medium enriched with 2 mM glutamine.

Endothelial cells were harvested from culture flasks by gentle rubbing with a polypropylene rod, and centrifuged at 100 ***g*** for 15 min at room temperature. The cells were resuspended in approximately 0·1 ml fresh culture medium and counted in a haemocytometer. The final suspension was frozen at −80°C and rapidly thawed at 37°C three times before analysis.

Immunofluorescence

Indirect immunofluorescence staining was performed by methods similar to those described by Giddings *et al.*[10] Briefly, frozen sections of the skin biopsy were treated with monoclonal antibody to vWFAg followed by fluorescein (FITC)-conjugated anti-mouse immunoglobulin. In some experiments, sections and coverslip preparations of endothelial cells in culture were treated with polyclonal (rabbit) anti-vWFAg together with rhodamine (TRITC)-labelled anti-rabbit immunoglobulin. All fluorescence experiments were controlled with appropriate non-immune serum and with immunoglobulin fractions of serum from the respective species.

RESULTS

The presence of von Willebrand factor antigen (vWFAg) was confirmed in frozen, histological

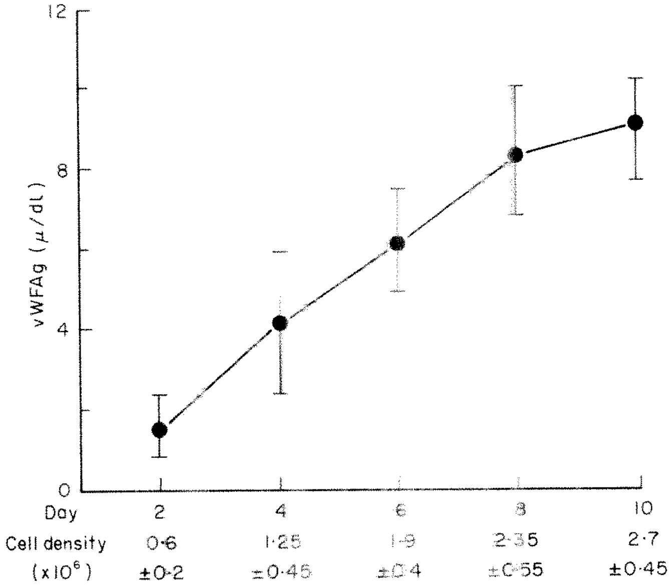

FIGURE 1. Relationship between amount of vWFAg measured by IRMA and number of human umbilical vein endothelium cells in culture. $n = 7$; cell density ± SD.

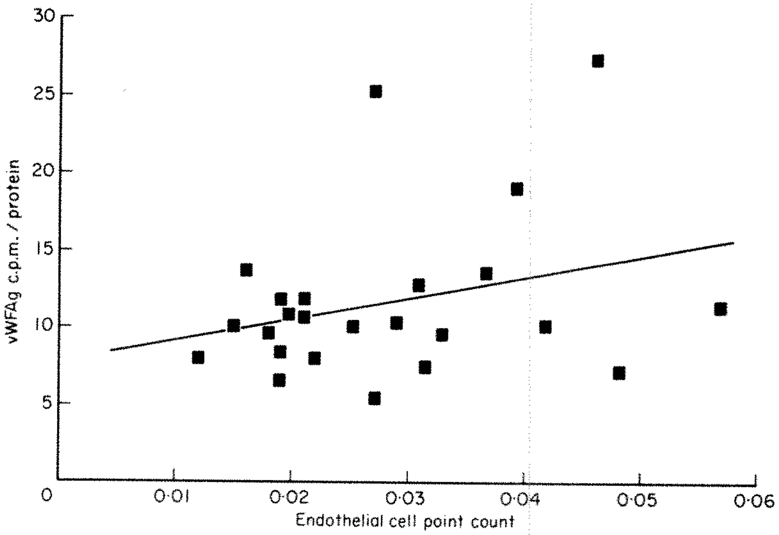

FIGURE 2. Relationship between vWFAg measured by IRMA and endothelial cell mass estimated stereologically in biopsies of normal skin, and the same site 3 days after biopsy. $r = 0{\cdot}30$; $P = 0{\cdot}16$.

TABLE 2. Relative volume endothelium ($V_{v,end}$) per unit volume dermis; and vWFAg (c.p.m./mg protein) in normal and wounded skin*

	Pre-wound	Post-wound
$V_{v,end}$	0·0196 ± 0·0048 (0·0191)	0·0363 ± 0·0106 (0·0348)
vWFAg	11·22 ± 4·85 (10·27)	12·12 ± 5·97 (10·53)

* Values are mean ± standard deviation (median).

sections of normal human skin and in the coverslip preparations of umbilical vein endothelium cells in culture using the indirect immunofluorescence method. The concentration of vWFAg in endothelial cells increased with time and was proportional to the number of cells harvested (Fig. 1). Investigation of the 3 mm punch biopsies of normal skin and of the 6 mm biopsies of wounds suggested that the concentration of vWFAg was related to the amount of endothelium present, measured stereologically (Fig. 2; Table 2). The figures illustrate radioactive counts per minute, relative to the protein concentration of the tissue sample. The data indicated that between 0·15 ng and 1·6 ng vWFAg was recovered in each tissue biopsy. Correlation between vWFAg and the volume of endothelium was not statistically significant ($P = 0{\cdot}16$) but the relationship was confirmed and extended in a study of 4 mm biopsies of skin from patients with psoriasis (Fig. 3). In these experiments, histometric evaluation of the tissues sampled demonstrated that the relative volume of endothelium per unit volume of dermis was greater in the involved than in the uninvolved areas of skin from the same patient taken at the same time.

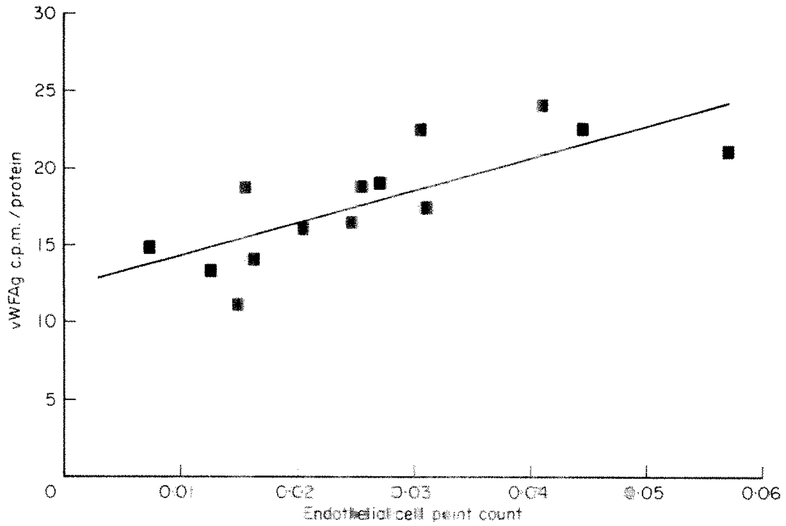

FIGURE 3. Relationship between vWFAg and endothelial mass estimated as for Figure 2 in biopsies of involved and uninvolved skin from patients with psoriasis. $r = 0{\cdot}775$; $P = 0{\cdot}001$.

TABLE 3. Relative endothelial volume ($V_{v,end}$) per unit volume dermis; and vWFAg (c.p.m./mg protein) in involved and uninvolved psoriatic skin*

	Involved	Uninvolved
$V_{v,end}$	0·0332 ± 0·0123 (0·0288)	0·0194 ± 0·0122 (0·016)
vWFAg	19·87 ± 2·84 (18·93)	15·88 ± 3·82 (14·42)

* Values are mean ± standard deviation (median).

Furthermore, the concentration of vWFAg was increased in the samples from involved skin compared to the uninvolved skin (Table 3) and there was a good overall statistical correlation between vWFAg and endothelial cell content ($r = 0{\cdot}775$). In all of these experiments the immunoradiometric assay for vWFAg was performed 'blind' and different personnel were involved in the two test systems.

DISCUSSION

The results of this study support the hypothesis that there is a linear relationship between the amount of vWFAg and the number of endothelial cells in the tissue. The endothelial cell contains the same amount of the antigen regardless of its age or other physiological variables. The data show that this is true for normal umbilical vein endothelial cells in culture and is probably also correct for normal endothelium *in vivo*. Our preliminary studies of biopsies of normal and wounded skin suggested that the concentration of vWFAg was related to the degree of vascularization assessed morphometrically, although in these early experiments the correlations did not reach statistical significance. This was possibly due to the small sample size and to the low numbers of blood vessels. In addition, granulation tissue in the wounded skin may have adversely affected the estimation of protein concentration. In contrast, a highly significant correlation between vWFAg and the volume of endothelium in skin biopsies from patients with psoriasis was observed. This may not be the case in diseased skin with abnormal endothelium; for example, in HIV infection in which patchy, irregular immunocytochemical localization of vWFAg has been found.[11]

It has also been suggested that endothelial cells during development and wound healing might be relatively deficient in vWFAg.[12] We have not been able to confirm this suggestion, and in the present immunofluorescence studies abundant quantities of vWFAg were observed in the capillary endothelium of wounded skin. This immunofluorescence technique was not quantitative, but three independent investigators each observed that the amount of antigen in wounded skin appeared to be at least as great as that found in normal skin. In addition, Magari[13] discussed the possible localization of vWFAg in Weibel Palade bodies of embryonic, lymphatic endothelium. We have failed, however, to detect vWFAg in adult lymphatic vessels and believe that in the current washed tissue biopsies vWFAg is located exclusively in the blood vessels.

It should be emphasized that it is unlikely that blood or platelet contamination contributed to the values of vWFAg determined by the assay because of the extensive wash procedures and the absence of albumin in the homogenate as determined by an ELISA assay.

The increased endothelial cell and vWFAg content of affected psoriatic skin compared with uninvolved skin of psoriatic patients is of interest but not unexpected.[14] It is not known whether this increase represents a primary alteration or is a secondary result of other tissue changes. It will be possible to study the effect of treatments for psoriasis on the endothelial component of the disease as well as to determine whether the increased content of endothelial cells persists in the uninvolved skin of patients with psoriasis compared with the skin of non-psoriatic individuals, using the immunoassay described.

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Investigation of the swollen limb with isotope lymphography

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SUMMARY

The usefulness of isotope lymphography was evaluated in patients with chronic lower leg swelling. Forty patients (15 male, 25 female, age range 5 months–67 years) were assigned one of six clinical diagnoses based on clinical assessments, venography and lymphography. All the patients and seven normal controls were examined with bilateral isotope lymphography involving subcutaneous injection of 0·15 ml ^{99m}Tc-antimony sulphide colloid into the first web space of each foot. The percentage uptake of isotope-colloid in the inguinal lymph nodes at 1 and 2 h was equated with lymphatic function. Highly significant ($P=0{\cdot}0001$) differences existed between the different clinical groups and isotope lymphography discriminated well between oedema of lymphatic and non-lymphatic origin. Isotope lymphography appears to be a safe and useful test of lymphatic function.

Chronic peripheral oedema frequently develops from either an excess of capillary filtrate with normal but overloaded regional lymphatics (venous hypertension, dependency syndrome) or from defective lymphatics with an unaltered lymph load (lymphoedema).[1] To distinguish chronic peripheral oedema of primarily lymphatic origin from other causes may not be straightforward. Investigation of the lymphatic system has relied solely on direct contrast lymphography, but this test is invasive and difficult to perform in the presence of oedema. Furthermore, it provides anatomical rather than functional information.

Lymphoscintigraphy was first introduced for imaging of lymph nodes.[2] Improvements in radiolabelled tracers have resulted in quantitative lymphoscintigraphy (isotope lymphography)[3] enabling measurement of limb lymphatic function. Previous authors have concentrated on cases of known lymphoedema where high specificity for lymphatic function has been demonstrated.[4–6] This study evaluated isotope lymphography as a test of lymphatic function in an assorted group of patients referred to the dermatology department with chronic lower leg swelling.

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PATIENTS AND METHODS

Forty consecutive patients with chronic oedema of the lower legs (15 male, 25 female; age range 5 months–67 years) and seven normal controls were examined with bilateral isotope lymphography. None of the patients or the controls had previous groin surgery. All patients were first assessed clinically by one physician (PSM) and the clinical diagnoses included: primary lymphoedema due to Milroy's disease (five patients, nine limbs); other causes of primary lymphoedema (13 patients, 19 limbs); secondary lymphoedema (seven patients, 10 limbs); mixed lymphovenous disease (eight patients, 14 limbs); recurrent cellulitis of unknown cause (three patients, four limbs) and venous disease alone (four patients, eight limbs). Most diagnoses were confirmed with venography and conventional lymphography. Where patients had unilateral disease, the 'normal' limb was also examined (16 limbs). The control subjects were members of hospital staff, or in-patients, with no evidence or history of oedema. In six patients, the investigation was repeated 2–12 months later to assess the reproducibility of the results.

Into the first web space of each foot was injected subcutaneously 0·15 ml of ^{99m}Tc-antimony sulphide colloid (Nanocoll, Solco-Nuclear, Basle, Switzerland). A large field-of-view gamma camera with on-line computer facilities was positioned with the collimator face close to the skin surface. Images were obtained sequentially over feet, knees and inguinal regions at 20 min, 40 min, 1 h and 2 h post-injection. The subject was exercised (normal walking) from 20 to 40 min after the injection.

The rate of clearance of the isotope-colloid from the site of injection, and the percentage uptake of isotope-colloid in the inguinal lymph nodes at 1 and 2 h were measured. The rate of clearance of isotope from the injection site was expressed as a percentage of the administered dose remaining, calculated from decay-corrected, time-activity curves for the injection site. For regional lymph node uptake of isotope, corrected time-activity curves were expressed as a percentage of the administered dose.

All subjects tolerated the procedure very well. The small injection was almost painless and no one experienced any side-effects. The measured radioactivity per injection was less than 20 mBq in all cases. This dose is within category I (the lowest range) of the classification used by the Administration of Radioactive Substances Advisory Committee (DHSS). Ethical approval for the study was obtained from the Wandsworth Health Authority District Medical Ethics Committee.

RESULTS

The dispersal of the isotope tracer from the site of injection along lymphatic channels to the regional lymph nodes could be visualized and anomolous drainage routes identified. Examples of isotope lymphogram images obtained at 2 h are given for a control subject, a patient with unilateral primary lymphoedema and a patient with venous disease (Figs 1–3).

The mean percentage uptake (± SE) of isotope-colloid in inguinal lymph nodes at 1 and 2 h was calculated for each group (Table 1). The 2-h uptake results were as follows. In Milroy's disease mean uptake (± SE) was 0·26% ± 0·067 (range 0·1–0·7%). In primary lymphoedema, excluding Milroy's disease, mean uptake was 0·92% ± 0·19 (range 1·13–3·1%). In secondary lymphoedema mean uptake was 0·98% ± 0·31 (range 0·14–2·73%). Thus, for all cases of primary or secondary lymphoedema mean uptake was 0·78% ± 0·13 (range 0·1–3·1%). In recurrent cellulitis the mean uptake was 2·77% ± 0·74 (range 1·2 ± 4·78). In mixed lymphovenous disease the mean uptake was 2·71% ± 0·55 (range 0·47 ± 7·93%). In venous disease alone

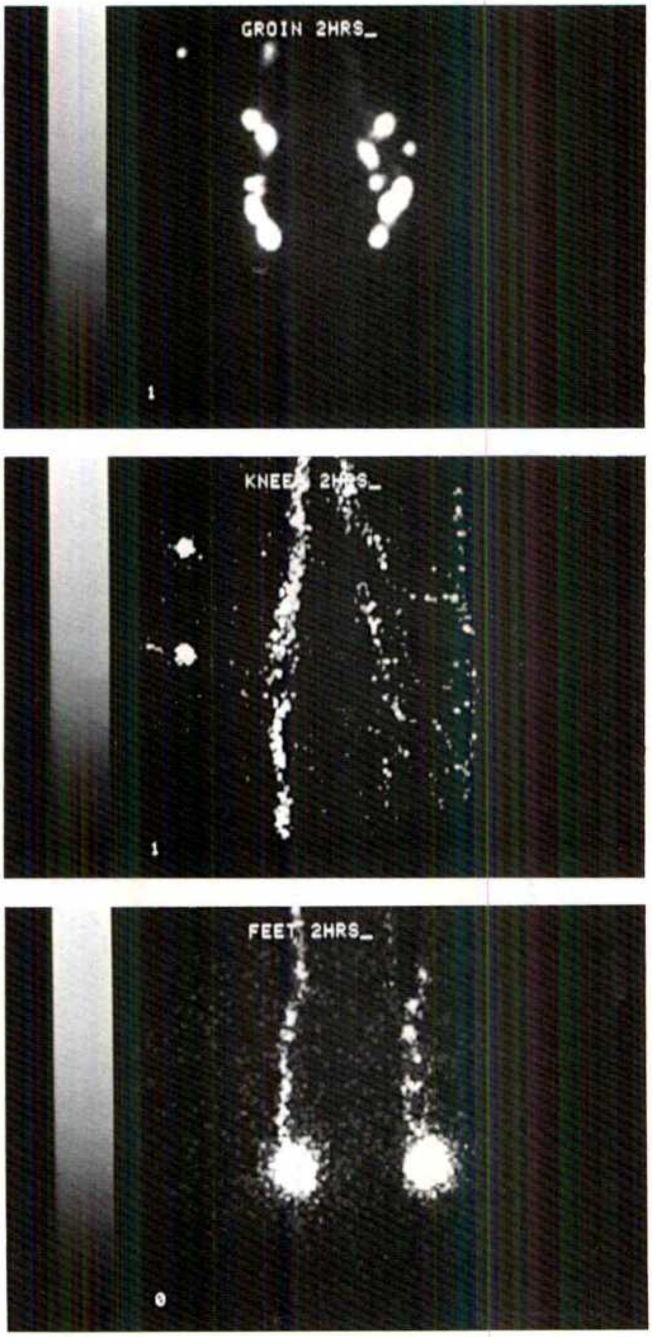

FIGURE 1. Image taken at 2 h, in a control subject, showing dispersal of isotope from the site of injection, anomalous lymphatic drainage around the left knee, and normal inguinal lymph node pattern.

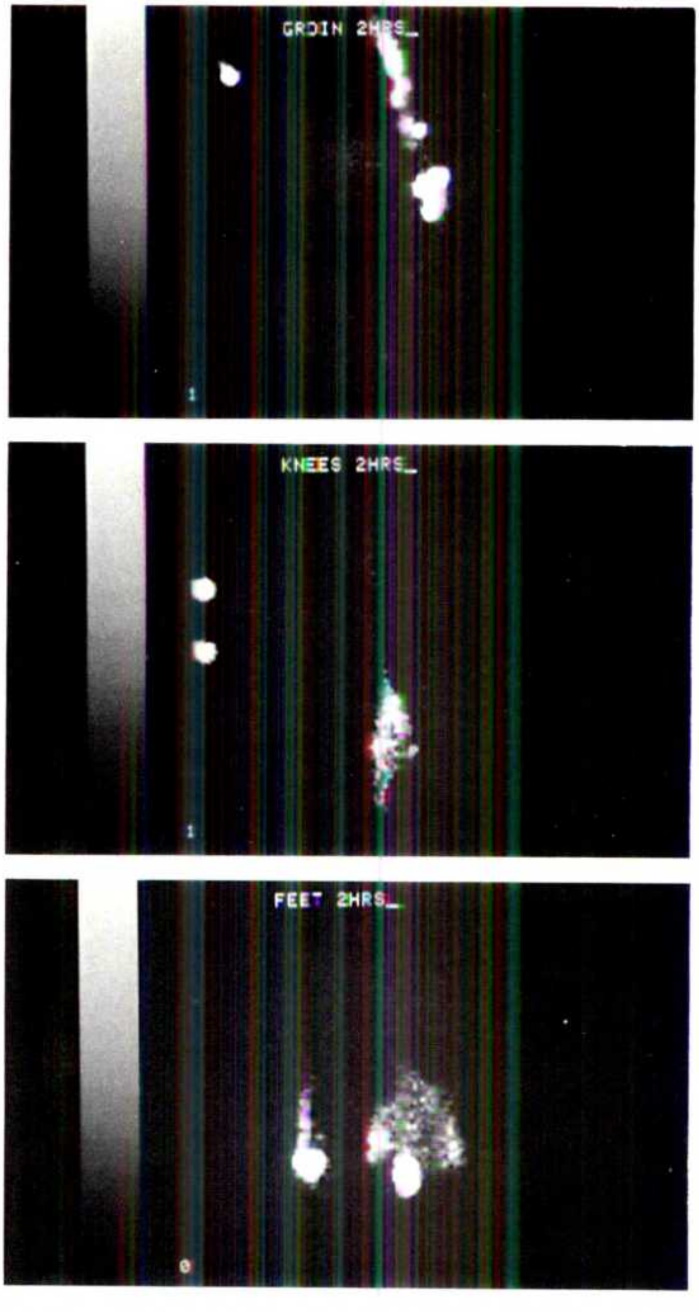

FIGURE 2. Image taken at 2 h, in a patient with unilateral Milroy's disease, showing relatively normal lymphatic drainage in the left leg but almost none in the right leg.

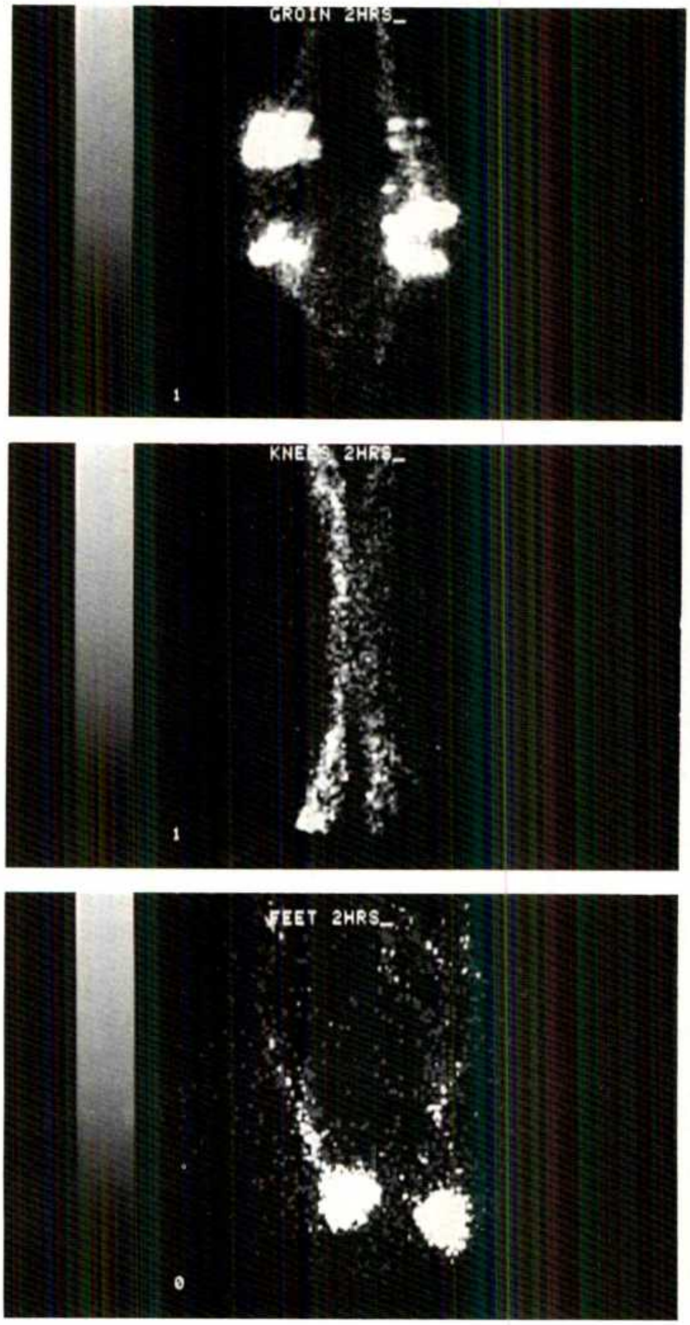

FIGURE 3. Image taken at 2 h, in a patient with predominantly venous disease showing good lymphatic function in both legs.

TABLE 1. The mean percentage uptake of isotope-colloid in inguinal lymph nodes at 1 and 2 h post-injection

Clinical subgroup	Number of limbs studied	Percentage uptake at 1 h (mean ± SE)	Percentage uptake at 2 h (mean ± SE)
Milroy's disease	9	0·19 (±0·035)	0·26 (±0·067)
Primary lymphoedema	19	0·54 (±0·16)	0·92 (±0·19)
Secondary lymphoedema	10	0·7 (±0·26)	0·98 (±0·31)
Recurrent cellulitis	4	1·42 (±0·42)	2·77 (±0·74)
Mixed lymphovenous disease	14	1·13 (±1·37)	2·71 (±0·55)
Venous disease alone	8	13·27 (±1·47)	19·33 (±1·39)
Normal legs in patients	16	9·85 (±1·32)	15·88 (±1·81)
Normal controls	14	11·08 (±1·53)	17·72 (±1·82)

the mean uptake was 19·33% ± 1·39 (range 14·7–26·2%). In normal controls the mean uptake was 17·72% ± 1·82 (range 8·25–28·89%) and in 'normal' legs in patients with unilateral disease the mean uptake was 15·88% ± 1·81 (range 8·0–29·6%). The overall analysis of variance within groups gives $P=0\cdot0001$. The *post hoc* Fisher's PLSD gives $P=0\cdot0001$ when comparing any of the five lymphoedema groups with venous disease alone, with normal controls, or with normal legs in patients. When the results from patients in the different clinical groups are shown graphically (Fig. 4), isotope lymphography can be seen to discriminate well between lymphoedema and oedema of venous origin.

The rate of clearance of the isotope-colloid from the site of injection was expressed as the percentage of the administered dose remaining, calculated from decay-corrected time-activity curves for the injection site. However, these measurements did not confer any additional advantage over uptake of isotope-colloid at regional lymph nodes. In almost all cases, percentage uptake of isotope-colloid in inguinal lymph nodes at 1 h gave results equivalent to those measured at 2 h. However, the interpatient variation within the clinical groups was wider when using uptake at 1 h. Thus, it was felt that isotope-colloid uptake at 2 h gave a more clinically helpful measurement of lymphatic function.

In the six patients in whom the procedure was repeated 2–12 months later, there was a good correlation between the two measurements in the same limb (correlation coefficient for raw data 0·85, $P=0\cdot0004$; correlation coefficient for log data 0·95, $P<0\cdot0001$) (Fig. 5). Lymphatic function is a dynamic process influenced by a variety of external factors which results in fluctuations from one moment to the next. The correlation between the degree of lymphatic impairment measured and the clinical severity of swelling in patients with asymmetrical limbs was 100%.

DISCUSSION

Isotope lymphography is easy to perform and was well tolerated by patients and control subjects alike. When used as a test of lymphatic function it clearly discriminated between patients with lymphoedema (primary or secondary) and normal controls and also those with venous disease alone. Patients with mixed lymphovenous disease or those with a history of recurrent cellulitis

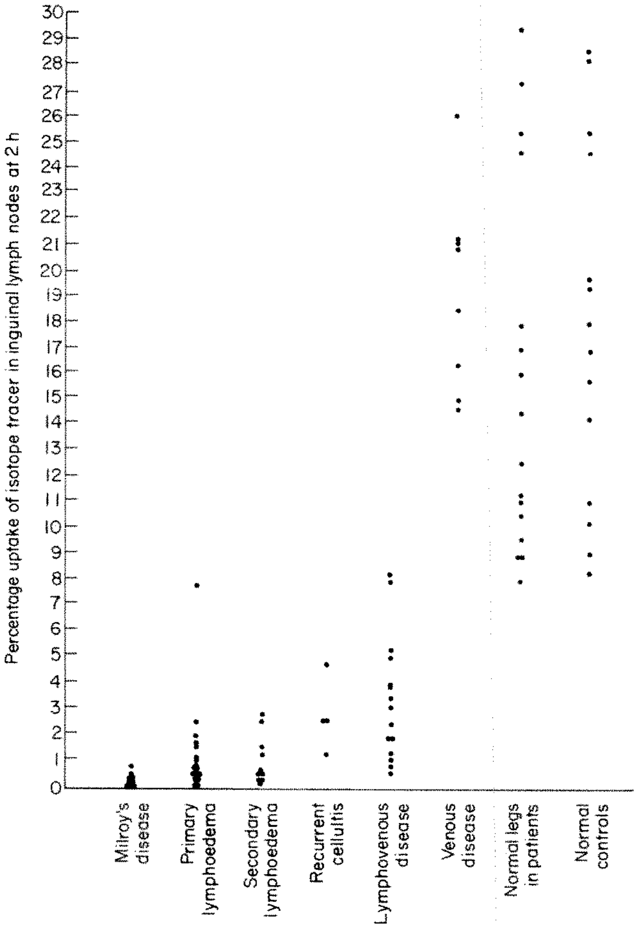

FIGURE 4. Isotope lymphography from the eight different clinical groups.

achieved values in between the other two categories, as would be expected with these conditions where there is a variable degree of lymphatic damage.

Milroy's disease is a primary lymphoedema resulting from absent cutaneous lymphatics. The access of isotope-colloid from the injection site to the peripheral lymphatics was grossly impaired, giving a characteristic picture at the injection site and virtually no clearance of tracer. No patient with Milroy's disease recorded more than 0·7% isotope tracer reaching inguinal lymph nodes after 2 h compared with control values of 8–29%. The degree to which lymphatic function was reduced in lymphoedematous limbs correlated well with the clinical severity of the lymphoedema. A fairly wide range of lymphatic function was measured in patients with primary lymphoedema other than Milroy's disease, and this was matched by the considerable variation in clinical severity seen amongst this group of patients.

Clinical signs of lymphoedema are frequently found in the lower leg and forefoot of patients with chronic venous ulceration. It is likely that lymphatic insufficiency is an important contributor to the chronic oedema seen in these leg ulcer patients although this has yet to be documented. All our patients with venous leg ulcers were included in the mixed lymphovenous

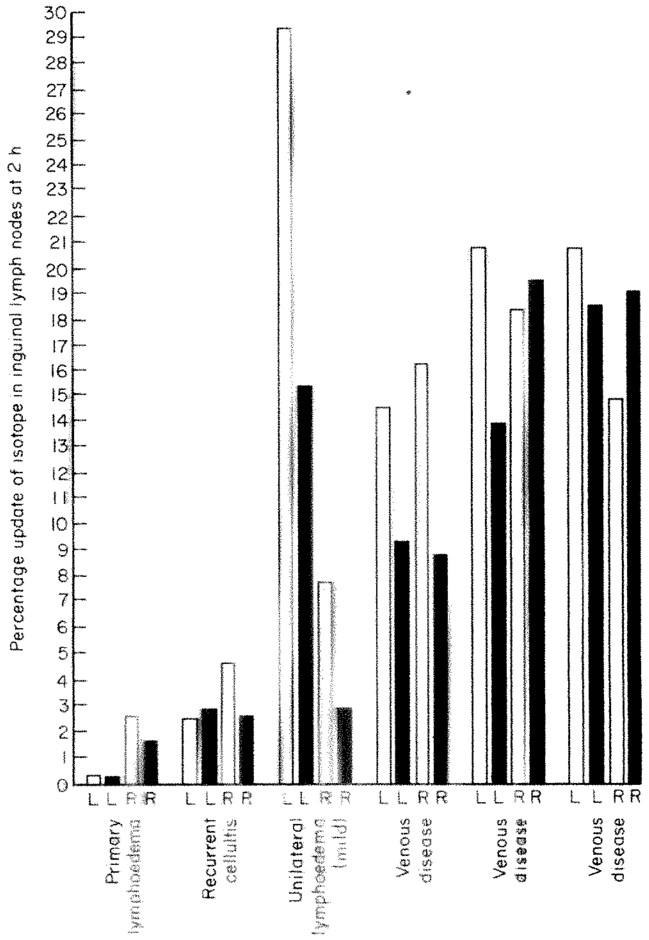

FIGURE 5. Reproducibility of results in six patients in whom isotope lymphography was repeated. □, first study; ■, second study.

group. Although the range of lymphatic reserve within this group will vary, isotope lymphography clearly separated these patients from those with venous disease alone. Thus, although lymph flow is probably increased in venous disease,[4] it seems that longstanding venous stasis leading to ulceration is associated with secondary lymphatic damage.

Exercise will contribute significantly to lymph flow. All our subjects were exercised from 20 to 40 min post-injection. This contrasts with the study of Stewart *et al.*[4] and may explain the high values achieved by some of our controls. Furthermore, we looked at 2-h uptake values compared with those at 30 min in their study. We found little relative difference between the clinical groups when comparing uptake results at 1 and 2 h, respectively. However, at 30 min we might have found significantly higher values in patients with venous disease compared with normal controls.

Isotope lymphography can distinguish primary lymphoedema from venous or lymphovenous disease. This avoids unnecessary investigations in patients with primary lymphoedema, although a full non-invasive venous assessment is always worthwhile. However, isotope lymphography does not indicate the cause of the lymphoedema. Lymphatic function may be equally impaired in lymphoedema due to pelvic obstruction, e.g. cancer, or from congenital insufficiency.

Lymphatic function provides a guide to prognosis since oedema of non-lymphatic origin will respond more rapidly and more completely to measures such as leg elevation and external support. Similarly, isotope lymphography theoretically provides a non-invasive means for monitoring progression of disease or for assessing improvement in patients receiving specific treatment for their limb oedema. It is a straightforward, safe and painless procedure.

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Deposition of C3, C9 neoantigen and vitronectin (S-protein of complement) in lichen planus pemphigoides

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SUMMARY

We have compared the distribution of C3, C9 neoantigen (C9n) and vitronectin at the dermo–epidermal junction in lichen planus pemphigoides with that in bullous pemphigoid. Eight out of 30 biopsies from patients with lichenoid lesions had linear C3 deposition at the basement membrane zone (BMZ); four of these patients had bullae and fulfilled the criteria for lichen planus pemphigoides. C9n immunoreactivity was detected as a linear or an intermittent linear/granular band at the BMZ only in these four patients, suggesting a role for the membrane attack complex of complement (MAC) in the pathogenesis of blister formation in lichen planus pemphigoides. Faint linear deposition of vitronectin, in addition to C9n, at the BMZ was seen in two of the four cases of lichen planus pemphigoides and three of six cases of bullous pemphigoid. This suggests that vitronectin may be deposited in association with C9n not only as part of the non-lytic SC5b-9 complex, but also as a regulatory step following the lytic action of MAC. A regulatory function for vitronectin in limiting tissue damage following activation of MAC is supported by our finding of a heavy deposition of vitronectin in association with C9n in a lichen planus pemphigoides patient in whom bulla formation had ceased.

Complement activation at the dermo–epidermal junction, seen in several important inflammatory dermatoses including bullous diseases, lupus erythematosus and lichen planus,[1] may lead to local damage by one of two methods. Binding of autoantibodies along the basement membrane zone (BMZ), as in bullous pemphigoid, leads to activation of the complement cascade, generation of fragments with chemoattractant properties, and tissue destruction mediated by enzymes released from attracted leucocytes.[2] Alternatively, complement activation

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on the keratinocyte cell surface may lead to cell lysis following assembly of the complement proteins C5–C9, formation of the membrane attack complex (MAC), and subsequent C9 polymerization.[3] Vitronectin, also called serum spreading factor, epibolin and S-protein of complement, is a multi-functional glycoprotein present in plasma, at cell surfaces, and in close association with the normal elastic tissue of adults.[4-7] Soluble vitronectin inhibits the MAC of complement by binding to nascent C5b–7 complexes, thereby blocking C9 polymerization and membrane lysis by formation of the so-called non-lytic SC5b–9 complex.[8,9] Formation of MAC by polymerization of C9 leads to the expression of C9 neoantigens (C9n) which can be detected using specific antibodies, but these do not distinguish between C9n in MAC and C9n in the non-lytic SC5b–9 complex.[10,11] C9n has been detected in lesional but not in uninvolved skin in lupus erythematosus,[12] and in greater quantities in lesional compared with uninvolved skin in bullous pemphigoid.[13] C9n immunoreactivity without detectable colocalized vitronectin along the BMZ has been reported in bullous pemphigoid;[14] this has been interpreted as evidence of MAC deposition in pemphigoid lesions. By contrast, it has been proposed that where there is colocalization of both C9n and vitronectin, as at the BMZ in discoid lupus erythematosus and in the tips of dermal papillae in dermatitis herpetiformis, this may represent deposition of C9 as part of the non-lytic SC5b–9 complex rather than of MAC.[14] However, vitronectin has been found in association with cytolytic membrane-bound C5b–9 complexes,[15] and BMZ deposition of both MAC and vitronectin in epidermolysis bullosa acquisita has been claimed as evidence of activation of terminal complement components in this condition.[16]

The characteristic immunofluorescence findings in lichen planus include deposition of fibrin at the BMZ and of immunoglobulins and C3 on dermal keratin (colloid) bodies.[1,17] In the rare bullous variant termed lichen planus pemphigoides, there is linear BMZ deposition of IgG and C3 in perilesional skin.[17-25] Keratin bodies have been reported to stain with anti-vitronectin antibody,[7,26] but no information is available with regard to the presence of C9n and/or vitronectin in lichen planus pemphigoides. We have therefore compared the distribution of C9n and vitronectin in lichen planus pemphigoides with that in bullous pemphigoid, in an attempt to elucidate the relationship between these two disorders.

METHODS

Specimens of lesional skin from patients with lichen planus, lichen planus pemphigoides, bullous pemphigoid and cicatricial pemphigoid were studied (Tables 1 and 2). In all cases patients had characteristic clinical signs and histopathological changes, and in the bullous and cicatricial pemphigoid cases, also the characteristic immunofluorescence findings. Thirty biopsies from patients with lichen planus were investigated for diagnostic purposes at the Immunofluorescence Laboratory, Department of Dermatology, University of Innsbruck, over a 27-month period; eight were specifically selected for study, because in each case routine immunofluorescence revealed linear deposition of C3 at the BMZ. Of these eight patients, one (Case 3) had lesions confined to the oral mucosa, three (Cases 5, 6 and 7) had bullae on lesional skin (in Case 7 bullae also occurred on skin sites without lichenoid changes), and one (Case 8) gave a history of blistering and oozing lesions, but at presentation had only a lichenoid eruption. Biopsies were taken from the oral mucosa in Case 3, from bullae in Cases 6 and 7, and from lichenoid lesions in the other cases. In the remaining 22 patients, no C3 was detected at the BMZ; one of these patients had extensive erosions on the feet, with histological features of bullous lichen planus.

Direct immunofluorescence was performed on all specimens by standard techniques using

TABLE 1. Linear BMZ deposition of C9 neoantigen or vitronectin in patients with lichenoid lesions and linear BMZ C3

Patient	Age	C3	C9n	Vitronectin	Bullae
1*	63	+ +	0	0	0
2*	42	+ + +	0	+ + +	0
3*	50	+ + +	0	Trace	0
4*	43	+ + +	0	0	0
5†	41	+ + +	+	Trace	+
6†	18	+ + +	Trace	0	+
7†	21	+ + (Gran)	+ +	Trace	+
8†	57	+ + +	+ +	+ + +	+

*Lichen planus on clinical and histological criteria.
†Lichen planus pemphigoides on clinical, histological and immunofluorescence criteria.

TABLE 2. Linear BMZ deposition of C3, C9 neoantigen or vitronectin in bullous and cicatricial pemphigoid

Patient	Age	C3	C9n	Vitronectin
1*	81	+ + +	Trace	0
2*	63	+ +/+ + +	+	0
3†	74	+ + +	+	0
4†	81	+ +/+ + +	+	0
5†	90	+ +	+	0
6†	85	+ + + +	+	Trace
7†	81	+ + +	+ +/+ + +	Trace
8†	47	+ + +	+ +/+ + +	Trace

*Cicatricial pemphigoid.
†Bullous pemphigoid.

fluoresceinated (FITC-) antibodies to human IgG, IgM, IgA, C3 and fibrinogen (Dakopatts, Glostrup, Denmark). For the detection of vitronectin, serial 4 μm cryostat sections were incubated with murine monoclonal antibody to vitronectin (Cytotech, San Diego, CA, U.S.A.) at a dilution of 1:50, and polyclonal rabbit anti-vitronectin at a dilution of 1:200, followed by FITC-sheep F(ab′)$_2$ anti-mouse IgG and IgM (Grub, Vienna, Austria) or FITC-swine anti-rabbit immunoglobulin (Dakopatts) at a dilution of 1:20, as appropriate. C9n was detected using polyclonal rabbit antibody to C9n[6] at a dilution of 1:50, followed by FITC-swine anti-rabbit immunoglobulin. Double-immunofluorescence staining experiments were also carried out using a 1:10 dilution of FITC-rabbit anti-human C3 (Dakopatts) followed by monoclonal anti-vitronectin and then a 1:40 dilution of rhodaminated (RITC-) F(ab′)$_2$ anti-mouse IgG and IgM (Tago, Burlingame, CA, U.S.A.).

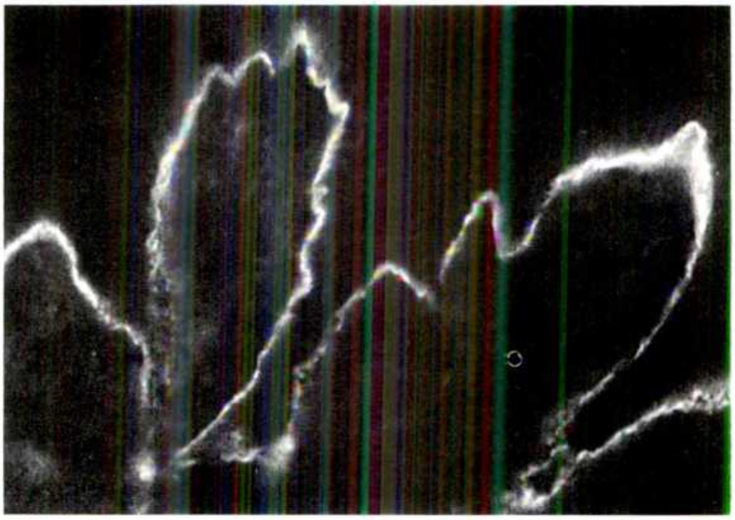

FIGURE 1. Linear immunofluorescence staining along the BMZ with anti-C3 antibody in lesional skin from a patient with lichen planus without bullae (Case 2) (× 220).

RESULTS

Strong linear C3 immunoreactivity at the BMZ was present in seven of the patients with lichenoid lesions (Table 1, Fig. 1), whilst in the remaining patient (Case 7) the C3 staining pattern was granular. Linear deposition of fibrinogen was detected in three patients (Cases 3, 7

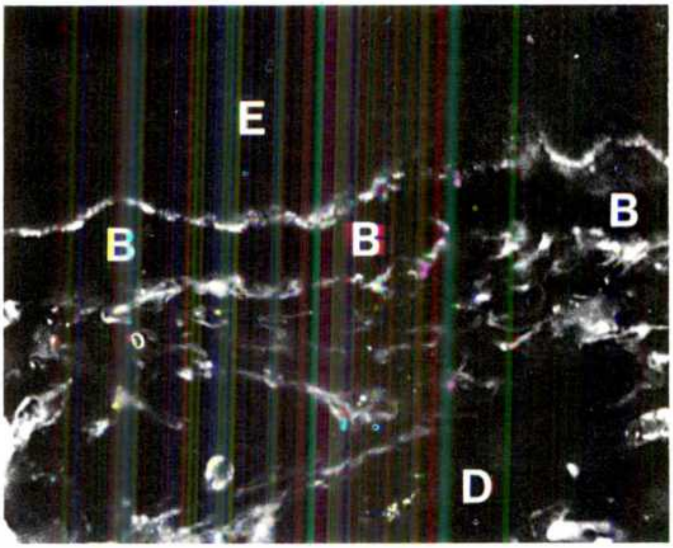

FIGURE 2. Linear immunofluorescence staining along the BMZ with anti-C9n antibody in lesional skin from a patient with lichen planus pemphigoides (Case 8). Note staining of elastic fibres. E, epidermis; B, blister cavity; D, dermis (× 220).

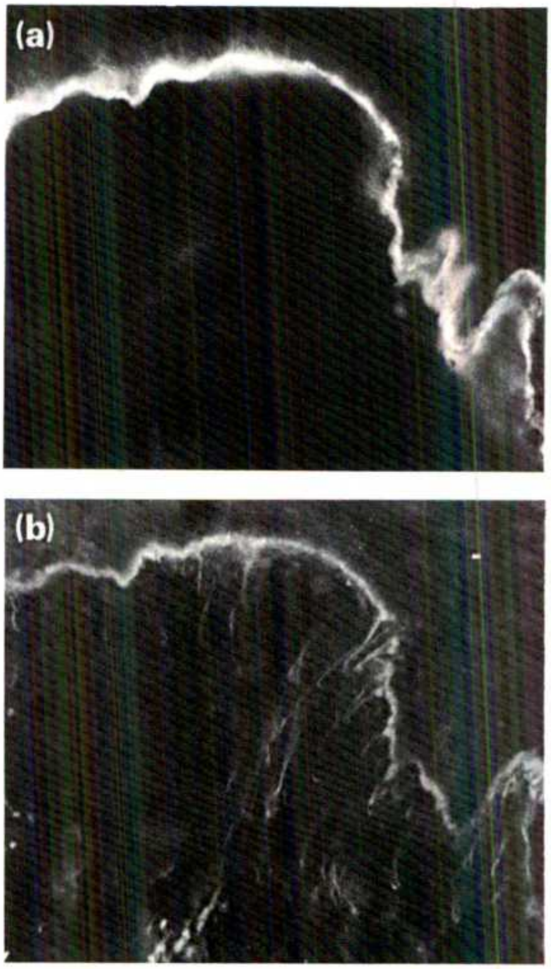

FIGURE 3. Double immunofluorescence staining of lesional skin from a patient (Case 8) with lichen planus pemphigoides using (a) FITC-anti-C3 and (b) monoclonal anti-vitronectin antibody followed by RITC-anti-mouse IgG/IgM. Note colocalization of linear fluorescence at the BMZ (× 220).

and 8), and linear IgG deposition in two patients (Cases 4 and 5). Clusters of dermal, and occasional epidermal, keratin bodies, which stained with anti-IgM, -IgA or -C3 antibodies, were found in six patients, and in two patients (Cases 3 and 7) C3 was deposited in and around dermal blood vessels. C9n immunoreactivity was detected as a linear (Fig. 2) or an intermittent linear/granular band at the BMZ only in the four patients (Cases 5–8) who had bullous lesions, and who fulfilled the clinical, histological and immunofluorescence criteria for lichen planus pemphigoides (Table 1). In several of the specimens, C9n immunoreactivity was also seen in association with dermal elastic fibres (Fig. 2), as previously reported.[6] In addition to the expected staining with anti-vitronectin antibodies of elastic fibres and keratin bodies in all biopsies, there was a strong linear band of vitronectin immunoreactivity at the BMZ (Fig.3) in two patients with lichenoid lesions (Cases 2 and 8), one of whom (Case 8) had bullae. Colocalization of vitronectin and C3 along the BMZ was demonstrated in both patients by

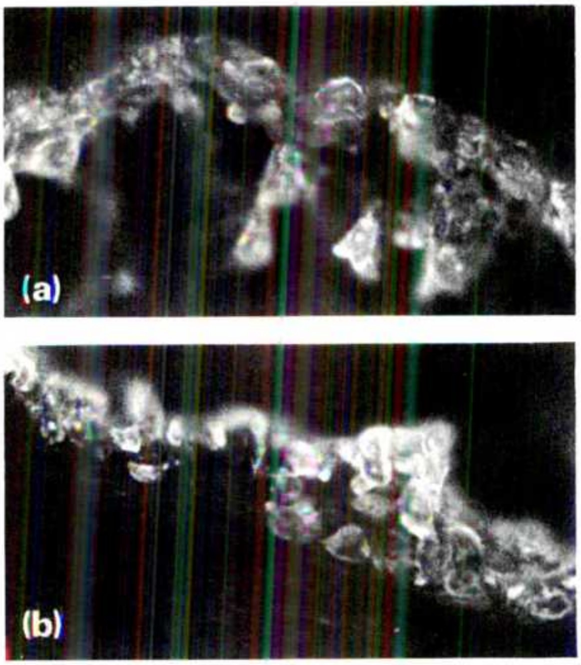

FIGURE 4. Immunofluorescence staining of lesional skin from a patient (Case 7) with lichen planus pemphigoides using (a) anti-C9n antibody and (b) anti-vitronectin antibody. Note bright fluorescence of basal keratinocytes in the roof of the blister with both antibodies (× 220).

double-immunofluorescence staining. Traces of vitronectin immunoreactivity were detected along the BMZ in a further three patients with lichenoid lesions (Case 3 without and Cases 5 and 7 with bullae). In Case 7, the keratinocytes of the basal layer of the epidermis, which formed the roof of a bulla, were heavily stained with anti-C9n and anti-vitronectin antibodies (Fig. 4), as well as with anti-IgM and anti-C3 antibodies.

Bright linear BMZ staining with anti-IgG, anti-IgA and/or anti-C3 antibodies was seen in all six cases of bullous pemphigoid and the two patients (Cases 1 and 2) with cicatricial pemphigoid (Table 2). C9n immunoreactivity was found as a linear, interrupted or dotted BMZ band in seven of the eight cases studied; in Case 1 only trace quantities of C9n were detected. Vitronectin immunoreactivity (using both monoclonal and polyclonal antibodies) at the BMZ was absent in five cases and present in trace amounts in the remaining three cases. Dermal elastic fibres stained in all cases with anti-vitronectin antibodies as expected, and in several cases also stained with anti-C9n antibody.

DISCUSSION

There is controversy in the literature as to whether lichen planus pemphigoides constitutes a distinct immunological entity, or whether it represents the coexistence of lichen planus and bullous pemphigoid.[18-25] The level of cleavage within the BMZ, and the site of deposition of immunoreactants, is within the lamina lucida on immunoelectron microscopy of skin in lichen planus pemphigoides and bullous pemphigoid.[20,24,25] The majority of bullous pemphigoid sera contain an antibody directed against a 230 kDa antigen, and a minority have antibodies which bind to a 180 kDa antigen.[27,28] Lang *et al.* reported that circulating anti-BMZ antibodies in a patient with coexisting lichen planus and bullous lesions reacted in an immunoprecipitin assay with an antigen different from those found in bullous pemphigoid.[22] However, Stanley *et al.* used serum from this patient, together with serum from two further patients with lichen planus pemphigoides, and found all three to contain circulating anti-BMZ antibodies directed against a bullous pemphigoid antigen.[25] Antibodies in the serum of a patient with drug-induced lichen planus pemphigoides immunoprecipitated a protein identified as bullous pemphigoid antigen.[29] Davis *et al.* reported binding of a serum antibody to an epidermal antigen of 180 kDa in two of three cases of lichen planus pemphigoides studied; in one there was additional binding to a novel 200 kDa antigen.[24] These findings would be compatible with either coexistence of lichen planus and true bullous pemphigoid in the same patient, or else with the concept that the inflammatory process in lichen planus may result in antibody formation to unmasked BMZ antigens, with deposition of immunoreactants at the BMZ and bulla formation occurring as a secondary phenomenon.[18,25]

In an attempt to elucidate the relationship between the two conditions, we compared the distribution of immunoreactants along the BMZ in bullous pemphigoid and in lichen planus pemphigoides. In order to identify patients with lichen planus pemphigoides for this study, we screened all biopsies from patients with a clinical diagnosis of lichen planus in whom material had been submitted for immunofluorescence over a 27-month period. One striking finding of this study was the high frequency of linear C3 deposition at the BMZ in lichen planus (eight out of 30 cases). Whilst four of these cases with linear BMZ C3 deposition either had bullae at presentation or a history of bullae, and could properly be classified as lichen planus pemphigoides, the remaining four cases had no evidence of bullae. Although linear BMZ deposition of immunoreactants in uncomplicated lichen planus has been previously reported,[30] the reason for the high frequency of this phenomenon in our series is not clear. This may reflect inclusion of the relatively more severe cases to be expected at a large tertiary referral centre.

It is of interest that, of the eight patients with lichenoid lesions and linear BMZ C3 deposition, bullae occurred only in the four cases in which linear C9n deposition was also present, suggesting a role for MAC in the pathogenesis of blister formation in lichen planus pemphigoides. Bullae in bullous lichen planus without BMZ deposition of immunoreactants may occur by a separate mechanism involving cell-mediated lysis of basal keratinocytes. It is also of note that linear deposition of vitronectin (faint in two cases and clear in another) in addition to C9n at the BMZ was seen in three of these four lichen planus pemphigoides cases with bullae. Moreover, three of the cases of bullous pemphigoid had vitronectin deposition, albeit faint, at the BMZ as well as C9n. This tends to confirm that colocalization of C9n and vitronectin does not necessarily mean that the C9n has been deposited as part of the non-lytic SC5b-9 complex and it is possible that the association occurs as a consequence of vitronectin deposition as a regulatory protein following the lytic action of MAC. This is well illustrated in Case 7 (lichen planus pemphigoides), in whom there was bulla formation associated with marked deposition of both C9n and vitronectin on basal keratinocytes. The presence of heavy staining with anti-vitronectin antibody in addition to C9n staining in Case 8 (lichen planus pemphigoides), with a history of blistering but in whom bulla formation had ceased by the time of biopsy, also suggests that vitronectin may have a regulatory function in limiting the extent of blistering.

Our findings that a similar pattern of deposition of immunoreactants may occur in both lichen planus pemphigoides and bullous pemphigoid does not resolve the issue as to the nature of their association. However, the fact that linear C3 and/or IgG deposition along the BMZ may occur even in cases of lichen planus apparently uncomplicated by blistering is perhaps more in keeping with the theory that linear BMZ deposition of immunoreactants in lichen planus pemphigoides occurs as a secondary phenomenon to damage at the dermo–epidermal junction. It would also suggest that lichen planus pemphigoides may simply be at one end of a spectrum of clinical presentations of lichen planus, comprising classical lichenoid lesions without deposition of BMZ immunoreactants; lichen planus without BMZ immunoreactants, in which bullae may occur as a result of oedema and keratinocyte destruction (bullous lichen planus); lichenoid lesions with immunoreactants but no bullae; and frank lichen planus pemphigoides with the immunofluorescence features typical of bullous pemphigoid.

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Association of EGF receptor expression with proliferating cells and of *ras* p21 expression with differentiating cells in various skin tumours

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SUMMARY

The localization of DNA replicating cells, epidermal growth factor (EGF) receptor-expressing cells and *ras* oncogene product p21 (p-21^{ras}) positive cells were examined in various skin tumours to elucidate the role of EGF receptor and $p21^{ras}$ in the epidermis. Normal skin, keratoacanthoma (KA), solar keratosis (SK), Bowen's disease (BD), squamous cell carcinoma (SCC), basal cell carcinoma (BCC) and extramammary Paget's disease (PD) were studied. EGF receptors were seen in proliferating layers, where DNA replicating cells localize, but $p21^{ras}$ was found in the more differentiated layers. We conclude that EGF receptor expression is closely associated with cellular proliferation, but $p21^{ras}$ may play a role in the differentiation of cells in various skin tumours.

Epidermal growth factor (EGF) is a potent mitogen for a variety of mammalian epithelial tissues and cells. EGF binds to a specific cell-surface receptor, the EGF receptor, and initiates a wide series of biochemical events leading on to mitosis and cell proliferation.[1] EGF receptors are found in the keratinocytes, especially the actively proliferating basal cells of the normal epidermis.[2–4] A high level of EGF receptor expression has been reported in various malignant tumours,[5] such as breast,[6] lung,[7,8] brain[9] and oesophagus.[10]

The human *ras* oncogene family has been identified as a normal cellular component and codes for the 21 kDa protein $p21^{ras}$ located on the inner surface of the plasma membrane.[11,12] $p21^{ras}$ appears to transmit receptor-mediated signals into the cytoplasm by binding guanosine triphosphate (GTP).[13,14] *Ras* genes are known to be activated by single-point mutations in

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some solid tumours[15,16] and in leukaemia.[17] It is also known that the *ras* gene, expressed at a high level, can transform NIH 3T3 cells.[18,19] Thus, not only qualitative changes but also an overexpression of *ras* gene are associated with cellular transformation. Many studies have shown that expression of $p21^{ras}$ is increased in carcinomas of various tissues, such as colon,[20] breast,[21] bladder,[22] ovary,[23] stomach[24,25] and prostate.[26]

We previously examined the distribution of EGF receptor-expressing epidermal cells and DNA replicating cells in psoriasis and Bowen's disease, and reported that the increased production of EGF receptors was partly responsible for the hyperproliferative state of the epidermis.[2] We have now extended the study to clarify the relationship between EGF receptor or $p21^{ras}$ and cellular proliferation in various skin tumours.

METHODS

Antibodies

BrdU and anti-BrdU antibodies were obtained from Ikeda Rika (Osaka, Japan) and Becton Dickinson (Mountainview, CA, U.S.A.), respectively. B4G7, mouse monoclonal IgG antibody specific to the human EGF receptor,[27] and NCC-RAS-001, antibody to *ras* oncogene product p21,[28] were described previously. Vectastain avidin-biotin-complex (ABC) kit and biotinylated horse anti-mouse IgG were obtained from Vector (Burlingame, CA, U.S.A.), OCT compound from Miles Laboratory (Naperville, IL, U.S.A.), normal mouse IgG and 3,3′diaminobenzidine (DAB) from Sigma (St Louis, MO, U.S.A.).

Skin specimens

Skin specimens were taken under local anaesthesia from 10 patients with solar keratosis (seven hypertrophic and three acantholytic) (SK), eight patients with Bowen's disease (BD), six patients with squamous cell carcinoma (SCC), 10 patients with basal cell carcinoma (BCC), three patients with keratoacanthoma (KA), five patients with extramammary Paget's disease (PD) and 10 normal individuals (five from the sole of the foot and five from the back).

BrdU staining

DNA replicating cells were detected by the ABC method using anti-BrdU monoclonal antibody after incubating tissues with BrdU as previously described.[2] In brief, 1 mm sliced tissue samples were incubated with 400 μM BrdU in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal calf serum (FCS) at 37°C under 3 atmospheric pressures for 2 h, fixed in 70% ethanol and embedded in paraffin. The paraffin was removed from the sections which were immersed in 2 M HCl for 30 min, and neutralized for 10 min in 0·1 M $Na_2B_4O_7$. They were then incubated at 4°C overnight with anti-BrdU monoclonal antibody as a first antibody, and subsequent steps were carried out according to the ABC staining method. The sections were stained with 3,3′diaminobenzidine (DAB) (0·5 mg/ml) in the presence of 0·01% H_2O_2 and counterstained with methyl green or eosin. Normal mouse IgG was used as a negative control. The labelling index was calculated as before[2] (2000 nuclei of epidermal cells were counted and classified as labelled or unlabelled).

EGF receptor staining

EGF receptors were detected by the ABC method using B4G7 mouse anti-human EGF receptor monoclonal antibody as previously described.[2,27] The tissues were embedded in OCT compound, snap frozen and 6 μm cryostat sections were cut in a cryostat. The sections were then fixed in ice-cold acetone, incubated with B4G7 antibody (1 μg/ml) in phosphate-buffered saline for 30 min at room temperature, and then treated with biotinylated anti-mouse IgG antibody.

Ras p21 staining

Ras oncogene product p21 was detected by the ABC method using NCC-RAS-001 mouse anti-human monoclonal antibody.[28] Tissues were fixed in 10% formalin or 70% ethanol and then embedded in paraffin. The paraffin was removed from the sections which were incubated with NCC-RAS-001 antibody at 4°C overnight and treated with biotinylated anti-mouse IgG antibody.

RESULTS

Table 1 summarizes the distribution of DNA replicating cells, EGF receptor-expressing cells and $p21^{ras}$ positive cells. In each skin condition the pattern of the distribution was the same for all cases examined, with little variability of staining intensity, except for the distribution of $p21^{ras}$ in the extramammary Paget's cells.

Distribution of DNA replicating cells

Cells that incorporated BrdU into DNA were detected by immunohistochemical staining using anti-BrdU monoclonal antibody. The DNA replicating cells were localized exclusively in the basal cell layer in normal epidermis (Fig. 1). In lesions of SK, DNA replicating cells were found only in the basal cell layer, although the number of labelled nuclei was five times greater than that of normal skin. In lesions of BD, DNA replicating cells were found in all the viable epidermal layers and the labelling index was the highest among the skin tumours examined. In lesions of SCC, DNA replicating cells were found in the mid to outermost layers of the tumour masses. The labelling index of SCC was 15·5% when all the tumour cells were counted, although it reached 25% when invasive areas were counted. Labelled nuclei were rarely found in those portions of the tumour masses that were keratinized or in those areas that were well

TABLE 1. Distribution of DNA replicating cells (labelling index), EGF receptor and $p21^{ras}$

	Normal epidermis (*n* = 10)	Kerato-acanthoma (*n* = 3)	Solar keratosis (*n* = 10)	Bowen's disease (*n* = 8)	Squamous cell carcinoma (*n* = 6)	Basal cell carcinoma (*n* = 10)	Extramammary Paget's disease (*n* = 5)
DNA replicating cells	Basal cell layer	Outermost layers	Basal cell layer	All viable epidermal layers	Mid to outermost layers	Mostly in outermost layers	In Paget cells
(LI)	2·5 ± 1·0	5·0 ± 1·0	12·7 ± 3·8	17·0 ± 5·5	15·5 ± 3·2	10·9 ± 5·2	12·5 ± 1·0*
EGF receptor expression	Basal and suprabasal layers	Mid to outermost layers	Basal and suprabasal layers	All viable epidermal layers	Mid to outermost layers	All portions of tumour masses	Negative
ras p21 expression	Upper squamous layers	Mid to innermost layers	Upper squamous layers	Mid to upper squamous layers	Innermost layers	Negative	Mostly negative

LI, labelling index (average ± SD).

* Labelling index in Paget cells = number of labelled Paget cell nuclei counted.

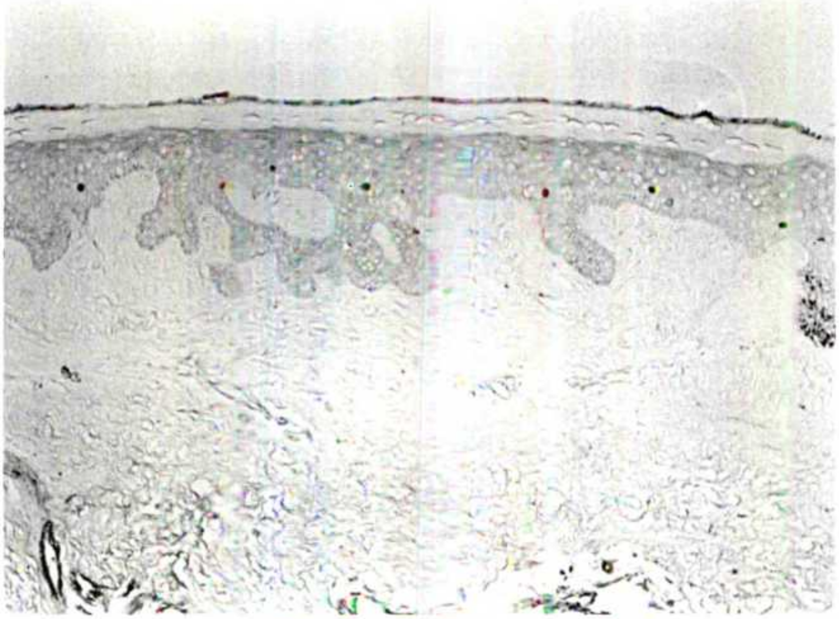

FIGURE 1. DNA replicating cells in normal skin (× 60).

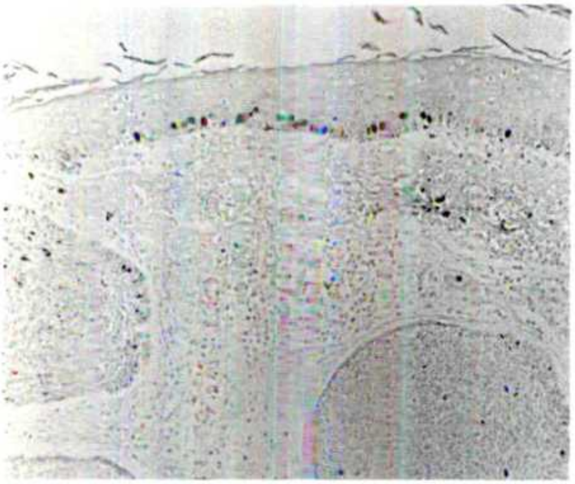

FIGURE 2. DNA replicating cells in the epidermis above the tumour masses of basal cell carcinoma (× 100).

differentiated. In lesions of BCC, DNA replicating cells were found mainly in the outermost layers of the tumour masses, though some labelled nuclei were found in the more central portions of the tumours. The epidermis above the BCC contained more DNA replicating cells in the basal cell layer than the adjacent normal appearing epidermis (Fig. 2). In lesions of KA, DNA replicating cells were found only in the peripheral portions of the tumour masses and the

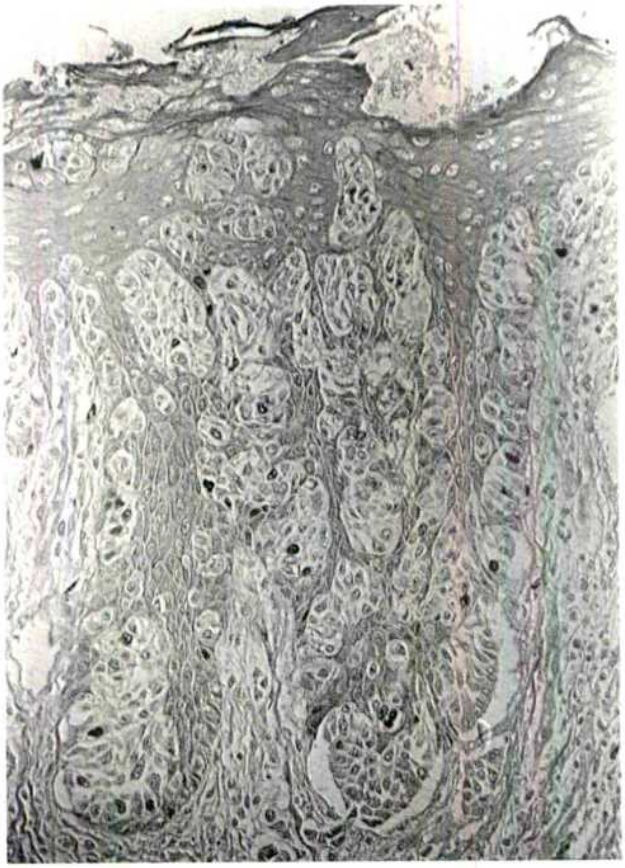

FIGURE 3. DNA replicating cells in Paget's disease (× 250).

labelling index was lower than that of SCC. In lesions of PD, the DNA replicating cells were found also in the Paget cells (Fig. 3). The surrounding epidermal cells had a normal labelling index and there was a normal distribution of DNA replicating cells.

Distribution of EGF receptors

EGF receptors were visualized by the anti-EGF receptor monoclonal antibody B4G7.[29] The EGF receptor-positive cells generally showed staining of the cell membrane with weak staining of the cytoplasm. In the normal skin, EGF receptor expression was seen mostly in the basal and suprabasal cells and this gradually diminished towards the upper part of the epidermis. In SK, EGF receptor expression was seen mostly in the basal and suprabasal cell layers as in normal skin, and in BD the tumour cells in all the layers of the epidermis were stained (Fig. 4). In SCC, EGF receptor was found in almost all the invasive portions of the tumour masses, but was not detected in the upper squamous cell layers. The basaloid cells in BCC were stained and the overlying epidermis above the tumour masses showed more intense staining compared with that of the adjacent epidermis. In KA, EGF receptor expression was observed in most of the tumour masses, but was not detected in the keratinized areas (Fig. 5). In PD, none of the Paget cells were stained but the normal appearing keratinocytes around these cells were stained (Fig. 6).

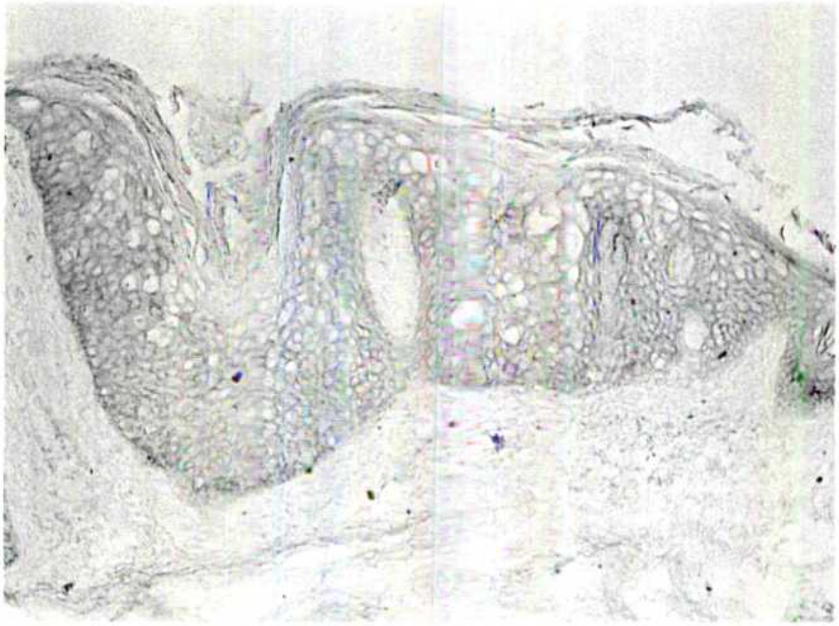

FIGURE 4. EGF receptor-expressing cells in Bowen's disease (× 60).

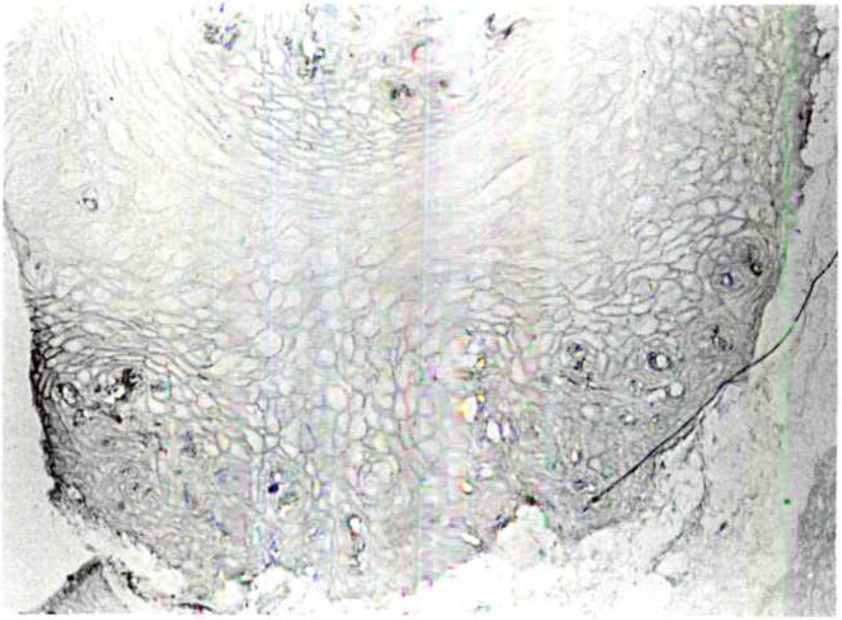

FIGURE 5. EGF receptor-expressing cells in keratoacanthoma (× 60).

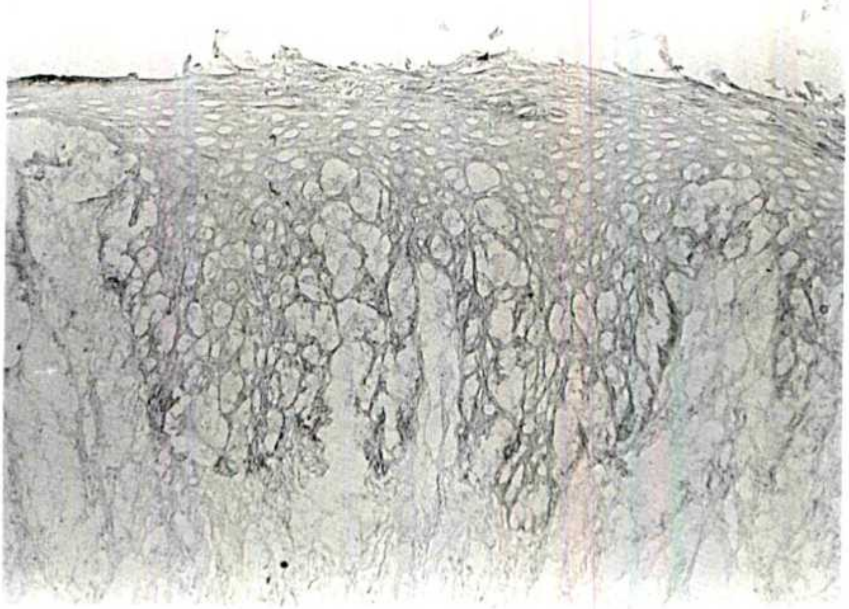

FIGURE 6. EGF receptor-expressing cells in Paget's disease (× 60).

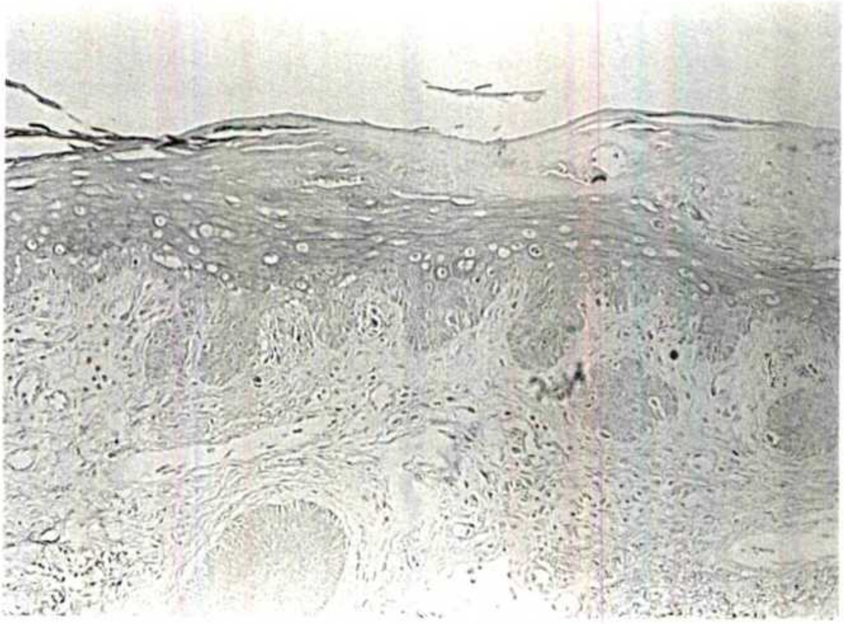

FIGURE 7. $p21^{ras}$ positive cells in solar keratosis (× 60).

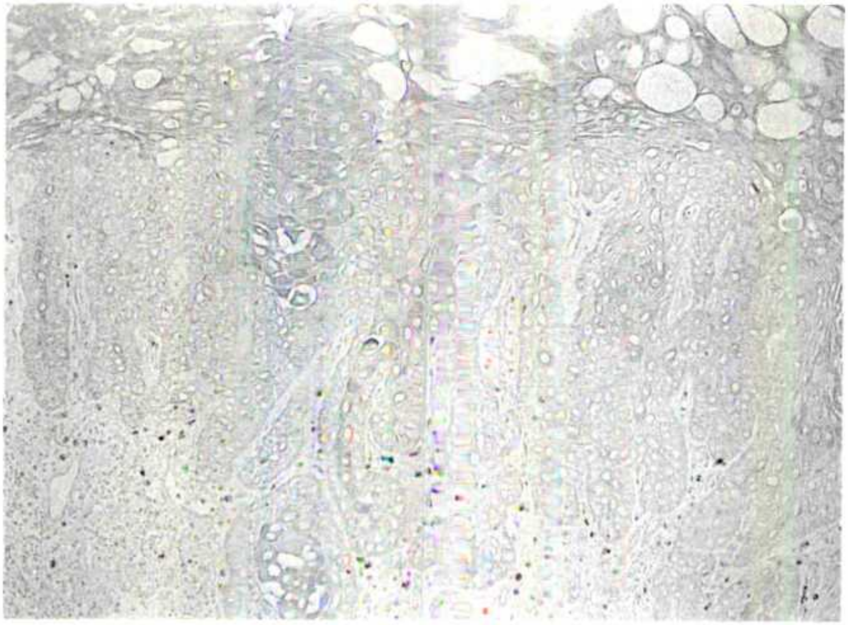

FIGURE 8. p21^{ras} positive cells in squamous cell carcinoma (× 60).

Distribution of ras p21

Ras oncogene product p21^{ras} was detected by immunohistochemical staining using the anti-p21^{ras} monoclonal antibody NCC-RAS-001[28] and was generally seen in the cytoplasm of the cells. In normal skin, p21^{ras} was expressed mostly in the upper squamous cell layers and was not visualized in the basal and suprabasal cell layers. In SK p21^{ras} was found in a similar location (Fig. 7). In BD the expression of p21^{ras} was in the upper squamous layers, but was also weakly positive in the mid-squamous layer. In SCC, p21^{ras} was found in the upper squamous layers (Fig. 8). In BCC the expression of p21^{ras} was not seen in the tumour masses, whereas in KA, it was detected in almost all parts of the tumour and the intensity was more than that found in SCC. In PD p21^{ras} was not usually found in the Paget cells but in a few cases was seen in the cells in the epidermis.

DISCUSSION

We have studied the localization of DNA replicating cells, EGF receptor-expressing cells and p21^{ras} positive cells to elucidate the relationship between EGF receptor or p21^{ras} and cellular proliferation in a number of different skin tumours.

In normal skin the basal cells undergo mitosis and then differentiate and the DNA replicating cells were to be found in the basal cell layer. The EGF receptor-expressing cells in normal skin were found in the basal and suprabasal cell layers and this suggests that EGF receptor is involved in the proliferation of epidermal cells. The p21^{ras} positive cells were found in the upper squamous layers where the cells are more differentiated and it is likely that the expression of p21^{ras} is not related to proliferation. These findings were seen in the various skin tumours studied. The distribution of EGF receptor-expressing cells was associated with that of the DNA replicating cells, whereas the distribution of p21^{ras} positive cells was not consistent with that of DNA replicating cells, but with that of more differentiated cells. EGF receptor expression is probably more closely related to cellular proliferation, whereas p21^{ras} may play a role in the differentiation of epidermal cells.

It has been reported that immunoreactive EGF receptors were found throughout psoriatic epidermis including the stratum corneum,[30] but in this study EGF receptor-expressing cells were not detected in the well-differentiated keratinized areas.

It has been suggested that the *ras* gene is involved in transformation in many human neoplasms, and that expression of p21^{ras} is necessary to initiate or promote this malignant transformation. [20-26] However, several studies have indicated that p21^{ras} expression does not relate to proliferation in normal human tissues,[31] keratoacanthomas[32] and pheochromocytomas.[33] Our unexpected finding that p21^{ras} relates to differentiation substantiates these studies.

In the epidermis above the tumour masses of BCC the number of DNA replicating cells was increased and EGF receptor expression was seen in all the viable layers. It may be that the proliferative state of the overlying epidermis is induced by a factor derived from the tumour cells. Alternatively the overlying epidermis plays a role in carcinogenesis and the development of BCC.

In PD, a number of labelled nuclei were present but EGF receptor was not detected in Paget cells, and p21^{ras} expression was found only in a few cases. These results suggest that the Paget cells have unique characteristics and a variety of cell types.

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Dominance of memory over naive T cells in contact dermatitis is due to differential tissue immigration

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SUMMARY

$CD4^+$ T cells include a naive ($CD4^+$, $CD45RO^-$, $CD29^-$, $CD45RA^+$) as well as a memory subpopulation ($CD4^+$, $CD45RO^+$, $CD29^+$, $CD45RA^-$). These subpopulations represent different stages in T-cell development and function. Recently, it has been shown that inflammatory and neoplastic $CD4^+$ T-cell infiltrates are dominated by the memory subpopulation, whereas both subpopulations are about the same size in the peripheral blood. This was thought to be the result of *in situ* maturation of naive into memory T cells. We analysed early positive patch-test reactions 1–2 days after antigen challenge and found that most of the $CD4^+$ T cells that had freshly immigrated into the tissue carried the memory phenotype. Their preferential migration may be mediated by at least five adhesion molecules expressed on their cell surface. This observation has important pathogenetic implications, since memory T cells can be rapidly activated by antigens and secrete a wide variety of pro-inflammatory cytokines.

It has recently become evident that $CD4^+$ T cells are heterogeneous both functionally and phenotypically.[1,2] Using monoclonal antibodies against several lymphocyte surface molecules (CD45RA/2H4, CD45RO/UCHL1, CD29/4B4, HB-11), two major $CD4^+$ T-cell subsets were characterized.[3–5] Morimoto and co-workers regarded these $CD4^+$ subpopulations as distinct and designated them as suppressor/inducer cells and helper/inducer cells according to their regulatory influence on B-cell immunoglobulin production.[4,5] An alternative model, now generally accepted, was proposed by Tedder *et al.*, in which the suppressor/inducer cells and helper/inducer cells represent sequential stages of maturation in T-cell development ('virgin' into 'memory' T cell).[3] This maturation is paralleled by fundamental changes in their functional properties.[3] Recently, the terms naive and memory T cells for these subsets were used and these have now been widely adopted.

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It has been demonstrated *in vitro* that CD4$^+$ naive T cells both phenotypically and functionally transform into CD4$^+$ memory T cells upon stimulation with phytohaemagglutinin,[6] ConA,[7] pokeweed mitogen[8] and alloantigen.[9] It is possible that *in vivo* a specific antigen will induce the transformation from the naive into the memory state. A reverse transition from the memory into the naive state has never been observed.[2] CD4$^+$ memory T cells are more effective than CD4$^+$ naive cells in amplifying an immune response, since they produce a greater proliferative response to stimuli binding either to the CD2 or CD3/TCR complex.[2] CD4$^+$ memory T cells secrete large amounts of γ-interferon, IL-3, and IL-4, which among other factors are responsible for maintenance and enhancement of an inflammatory tissue reaction. Also, the CD4$^+$ memory subset contains almost exclusively the cytotoxic activity of the CD4 population.[10] Thus, after an organism has had its first contact with an antigen, the immune system generates a subpopulation of CD4$^+$ memory T cells that can protect the organism during a second exposure to an antigen.

In the peripheral blood of healthy adults the two CD4$^+$ T-cell compartments are of similar size.[11] In contrast, a decrease of CD4$^+$ naive T lymphocytes was observed in various diseases such as rheumatoid arthritis or multiple sclerosis, both in the blood and in the inflamed tissues.[12-17] The dermal infiltrate of inflammatory skin diseases as well as malignant CD4$^+$ cutaneous T-cell lymphomas show similar changes.[15,16] This decrease of CD4$^+$ naive T cells could be due to a transformation into memory T cells in the inflamed tissue after contact with

TABLE 1. Specificity, source and tissue distribution of monoclonal antibodies used in this study

Ag Designation	MAb Used	Ag Distribution
CD1a	Leu6*	Langerhans cells, cortical thymocytes
CD2	Leu5*	All T cells
CD3	Leu4*	All T cells
CD5	Leu1*	All T cells
CD4	Leu3*	Memory (helper/inducer) T cells, naive (suppressor/inducer) T cells, activated macrophages
CD8	Leu2*	Suppressor/cytotoxic T cells
CD71	OKT9†	Transferrin receptor
CD25	Anti-tac*	Interleukin 2 receptor
CD30	Ki1	Activated T and B cells, Hodgkin and Sternberg–Reed cells
CD45RA	Leu18*	CD4$^+$ naive (suppressor/inducer) T cells, B cells (Leu18 detects the same antigen as 2H4 antibody)
CD45RO	UCHL1†	T cells; 30-fold higher expression on memory than on naive T cells, minority of monocytes
CD29	4B4‡	CD4$^+$ memory T cells, keratinocytes, broad variety of soft tissues
CD22	CD22†	B cells

* Becton Dickinson, Heidelberg, F.R.G., † Dakopatts, Hamburg, F.R.G.
‡ Coulter Electronics, Krefeld, F.R.G.

their respective antigens. However, it may be that CD4$^+$ memory cells preferentially leave the circulation at sites of inflammation. We have studied early positive epicutaneous patch-test reactions, to investigate the phenotype of lymphocytes leaving the circulation. Almost all CD4$^+$ T cells in the early patch-test reactions have the phenotype of memory T cells. This indicates that the predominance of CD4$^+$ memory T cells is due to preferential immigration of these cells into the inflamed tissue.

METHODS

Biopsy specimens

Punch biopsies (4 mm) were taken under local anaesthesia from four patients with early positive patch-test reactions; in one patient, two positive reactions with different allergens were biopsied ($n = 5$. Case 1, phenylephedrine; Case 2, eusolex; Case 3, bezocaine; Case 4a, eucerin; Case 4b, parabensmix). Informed consent was given. The patients had been referred to the Department of Dermatology for routine patch testing. All the biopsies were taken within 24 h after the antigen had been applied to the skin for 24 h. The biopsies were snap frozen in liquid nitrogen and 8 μm cryostat sections were prepared.

Staining procedures

The specificity and source of antibodies used in this study are shown in Table 1. Visualization of the antibody binding was performed using a three-step immunoperoxidase technique.[18]

Evaluation of slides

All stained slides were examined using light microscopy; 200 cells were evaluated and the percentage of positive (brown) and negative cells calculated.

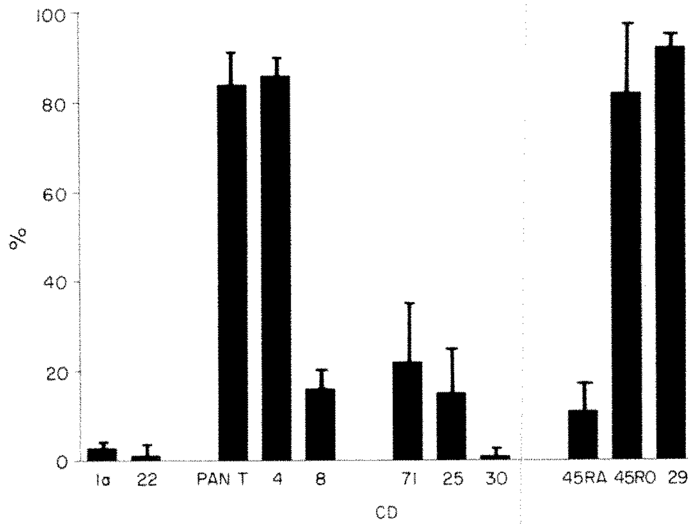

FIGURE 1. Mean values (per cent) with standard deviation of immunoreactivity in the dermal infiltrates of positive patch-test reactions. Pan T includes CD2, CD3, and CD5.

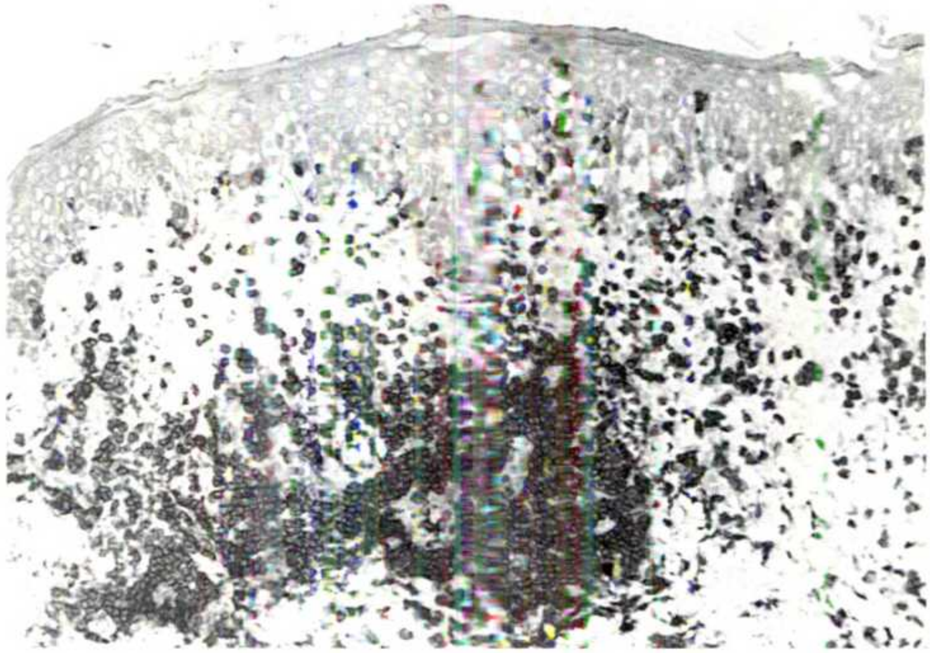

FIGURE 2. A positive patch-test reaction stained with UCHL1, a monoclonal antibody against the CD45RO antigen. Immunoperoxidase technique (magnification × 100).

RESULTS

The overall cellular composition was similar in all five biopsies. T cells ($CD2^+$, $CD3^+$, $CD5^+$) made up 80–90% of the infiltrate, the majority of them being $CD4^+$. $CD8^+$ T cells never exceeded 20% of all the cells. B cells occurred only sporadically, and $CD1^+$ Langerhans cells accounted for, at the most, only 5% of the dermal infiltrate (Fig. 1). The type of allergen did not influence the composition of the infiltrate. The activation antigen CD30 was rarely expressed (5% in one case), whereas the percentage of $CD71^+$ (transferrin-receptor) and $CD25^+$ (interleukin-2 receptor) cells varied from 5 to 40%. CD71 and CD25 expression paralleled each other.

Most of the cells in the infiltrate (Fig. 1) had the phenotype of memory T cells ($CD45RA^-$, $CD45RO^+$, $CD29^+$). All these cells strongly expressed CD45RO epitope (Fig. 2) and CD29 antigen.

DISCUSSION

The reliability of phenotypical identification of $CD4^+$ naive and memory T cells in tissue sections depends on the composition of the infiltrate. CD45RO/UCHL1 and CD29/4B4 are highly expressed on memory T cells,[19,20] and result in strong and reliable staining both in cell suspensions and tissue sections.[21] However, CD45RO/UCHL1 is also expressed on some monocytes and macrophages. In addition, CD29 is the common β-chain of the large β_1-integrin family and is found on nearly every cell type of the skin.[22] Moreover, if many B cells are present in an infiltrate (lymph node, spleen, etc.), CD45RA (2H4, Leu18) is of little value as a marker for the naive subset as it also detects B cells. Therefore, in mixed infiltrates double staining is necessary. However, in our study, as more than 80% of the infiltrate was made up by $CD4^+$ T cells an error due to overlapping subpopulations was unlikely.

We have shown that in allergic contact dermatitis (1–2 days after rechallenge with an allergen) there is a predominance of $CD4^+$ memory T cells in the $CD4^+$ infiltrate. As FACS-sorted naive T cells ($CD45RO^-$) stimulated with PHA need almost 7 days to acquire the features of memory T cells ($CD45RA^-/CD45RO^+$),[9,23] it is likely that this predominance is due not to local maturation, but to a preferential migration of $CD4^+$ memory T cells into the skin. $CD4^+$ memory T cells can interact with endothelial cells because they express at least five adhesion molecules on their surface (LFA-3, CD2, CD29, LFA-1, CD44), unlike naive T cells, which express these molecules only in low quantities.[2,19] LFA-3 on $CD4^+$ memory T cells will not mediate adhesion to endothelial cells, as its ligand CD2 is not expressed.[22] However, CD2 and LFA-1 (CD11a/CD18; αL β_2-integrin) might serve this function, as their ligands LFA-3 and ICAM-1 are constitutively expressed on skin endothelial cells,[22] and may be enhanced during inflammation by pro-inflammatory cytokines and bacterial products such as IL-1, TNF or LPS.[24] CD44, ELAM-1 and CD29 (β_1-integrin common β chain) may also participate, although their ligands remain to be identified.

It has been a matter of controversy as to how many of the T cells in contact dermatitis are antigen specific; because the patch test itself shows antigen specificity, at least some of the T cells must recognize the antigen and elicit the inflammatory response.[25] It seems likely that lymphocytes which are confronted with their corresponding antigen by antigen-presenting cells are fixed in the tissue, probably due to a transient increase of adhesiveness of LFA-1.[26]

In conclusion, we have shown that in allergic contact dermatitis the dominance of $CD4^+$ memory over naive T cells in the skin is not due to *in situ* maturation of naive into memory T cells, but rather reflects the selective migration of memory T cells into the dermis. This ability to leave the circulation is probably due to the adhesion molecules expressed on the surface of memory T cells. When their specific antigen is shown to memory T cells by antigen presenting cells in the skin, these T cells become fixed and activated, producing an inflammatory reaction.

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Vasoactive intestinal peptide (VIP) and peptide histidine methionine (PHM) in human eccrine sweat glands: demonstration of innervation, specific binding sites and presence in secretions

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SUMMARY

Vasoactive intestinal peptide (VIP) and peptide histidine methionine (PHM) immunoreactivities have been detected in alcohol extracts of human axillary skin using sensitive and specific radioimmunoassays. VIP immunoreactivity ($7{\cdot}63+2{\cdot}33$ pmol/g, $x+\mathrm{SE}$, $n=9$) was more abundant than PHM immunoreactivity ($3{\cdot}86+0{\cdot}56$ pmol/g, $x+\mathrm{SE}$, $n=9$). Immunocytochemistry of sections of skin revealed a network of VIP/PHM immunoreactive nerve fibres around the perimeter of eccrine but not apocrine sweat glands. *In vitro* autoradiography of skin sections using ^{125}I-labelled VIP and PHM, demonstrated binding sites on the membranes of eccrine gland secretory cells. The binding of each radiolabelled ligand was eliminated by the presence of a large molar excess of appropriate cold peptide but was unaffected when incubated with related peptide, indicating the presence of specific binding sites for both VIP and PHM. Radioimmunoassay of Sep-pak concentrated human sweat identified the presence of both VIP immunoreactivity ($30{\cdot}6$ pmol/l) and PHM immunoreactivity ($43{\cdot}4$ pmol/l). Reverse-phase HPLC analysis of axillary skin extracts and sweat, followed by radioimmunoassay of fractions, identified single resolved peaks of VIP and PHM immunoreactivity with identical retention times to synthetic standards. Eccrine sweat glands in human axillary skin have VIP and PHM peptidergic innervation and possess specific binding sites for each peptide which are both secreted to the surface of the skin.

Vasoactive intestinal peptide (VIP)[1] and peptide histidine methionine (PHM)[2] are structurally-related neuropeptides encoded by the same gene. One of the major biological effects of these peptides is their ability to stimulate exocrine secretions from a variety of tissues including the

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pancreas[3-5] and small intestine,[6,7] by a mechanism involving the binding to specific membrane receptors with subsequent stimulation of adenylate cyclase.[8] Both peptides have a widespread distribution in human tissues and each has been localized by immunocytochemistry to the periglandular nerves of eccrine sweat glands in the skin[9,10] In the present study, the levels of both peptides, assessed by specific radioimmunoassays, and their microanatomical distribution as assessed by immunocytochemistry were determined in human axillary skin, a region especially rich in eccrine sweat glands. In addition, *in vitro* autoradiography using ^{125}I-labelled peptides was performed to determine the localization and distribution of binding sites in skin sections. The levels of both peptides in the secretions from these glands were determined radioimmunometrically using exercise-induced sweat and immunoreactivity in these samples and in extracts of skin was characterized using reverse-phase HPLC.

METHODS

Tissue collection

Redundant fresh human axillary skin was obtained from patients who were undergoing elective surgical procedures for non-malignant and non-infective conditions. Following removal of the samples the subcutaneous fat was dissected free and, after a brief wash in ice-cold physiological saline to remove excess blood, the specimens were processed for peptide extraction and radioimmunoassay, immunocytochemistry of nerve fibres and *in vitro* autoradiography.

Tissue extraction and radioimmunoassay

Skin samples were snap frozen in liquid nitrogen and stored at −20°C prior to extraction. Frozen samples were weighed and macerated in 8 ml/g of ice-cold ethanol/0·7 mol/l HCl, 3 : 1, v/v. The extracts were then agitated on ice for 2 h and stored overnight at −20°C. The tissue suspensions were centrifuged for 30 min at 1500 ***g*** and the supernatants were dried using a stream of air. Following reconstitution in radioimmunoassay buffer (40 mmol/l sodium phosphate buffer, pH 7·2, containing 140 mmol/l NaCl and 0·2% (w/v) bovine serum albumin), the extracts were subjected to both VIP and PHM radioimmunoassays as previously described.[11,12] The PHM radioimmunoassay employed an antiserum to the analogous porcine peptide PHI which cross-reacts 75% with human PHM. To overcome this, synthetic human PHM standards were used in the radioimmunoassay and immunoreactivity was subsequently attributed to the human peptide and designated accordingly.

Immunocytochemistry

Skin samples (approximately 5 mm cubes) were fixed in 10 ml of an ice-cold, fresh solution of 0·4% recrystallized *p*-benzoquinone in phosphate-buffered saline (PBS), pH 7·2, in the dark for 2 h at 4°C. Tissues were removed from fixative solution and washed for 24 h in several changes of 5% (w/v) sucrose in PBS prior to cryoprotection in 30% sucrose (w/v) in PBS for 2 h. Cryoprotected tissues were then mounted onto cryostat stubs using Cryo-m-bed (Brights Instrument Co., Huntingdon, Cambs, U.K.) and orientated with the epidermis perpendicular to the cutting surface, before being frozen at −18°C. Sections (15 μm) were cut using a cryostat (Brights Instrument Co.) and were placed onto gelatine-coated coverslips before being air-dried at room temperature for 2 h. The sections were immersed in PBS for 1 h prior to immunostaining which was performed in a humid incubation chamber. Primary rabbit anti-VIP and PHM antisera were the same as those used in the radioimmunoassay. They were employed at a dilution of 1 : 200 in PBS containing 1% (w/v) bovine serum albumin and 0·3%

(v/v) Triton X-100. Each primary antiserum was incubated with tissue sections for 18 h at 4°C followed by washing in PBS. To increase the immunostaining intensity of peptidergic fibres, the sections were incubated a second time with primary antisera for 4 h at room temperature followed by buffer washing as previously described.[13] The sections were then incubated with biotinylated donkey anti-rabbit IgG (Amersham International, Amersham, Bucks, U.K.) for 30 min at room temperature followed by two 10-min washes in PBS prior to application of the final reagent, fluorescein-labelled streptavidin (Amersham International) for 30 min at room temperature. After a further two washes in PBS, the sections were mounted on glass microscope slides using Apathy's mounting medium (British Drug Houses Ltd., Poole, Dorset, U.K.) and examined by means of an Olympus BH 2 series incident fluorescence microscope (Olympus Optical, Tokyo, Japan) fitted with an EY 455 supplementary excitation filter. Stringent specificity controls were employed including omission of each sequential layer in the immunostaining reaction and preadsorption of each primary antiserum with a concentration range (10 ng–10 μg/ml diluted antiserum) of both synthetic VIP and PHM (Peninsula Laboratories, Merseyside, England).

In vitro autoradiography

Autoradiography was performed on tissue sections using modifications to the methodology described by Young and Kuhar[14] as reviewed by Palacios and Dietl.[15] Fresh axillary skin samples were placed into 10 ml of ice-cold 0·04% (w/v) paraformaldehyde in PBS for 4 h at 4°C to stabilize tissue proteins while preserving receptor-binding potential. Cryoprotection was achieved by immersion in ice-cold 30% (w/v) sucrose in PBS for 1 h. Specimens were mounted on cryostat stubs and 15 μm sections cut as described in the previous section. One hundred becquerels corresponding to 1·4 fmol of monoradioiodinated VIP and PHI (the porcine analogue of human PHM), purified using reverse-phase HPLC, were applied to the tissue sections in a volume of 100 μl of protease inhibitor cocktail. This was necessary to prevent degradation of the radioligand and contained HEPES (10 mmol/l), mannitol (300 mmol/l), bovine serum albumin (1%, w/v), bacitracin (1%, w/v), phosphoramidon (10 μmol/l), 1–10 phenanthroline (1 mmol/l) and phenylmethylsulphonyl fluoride (100 μmol/l) (Sigma Chemical Company, Poole, Dorset, U.K.). The tissue sections were incubated with radioligands for 1 h at room temperature in a humid chamber followed by two 10-min washes in ice-cold PBS prior to fixation of radioligands to binding sites using a 30 min immersion in 4% paraformaldehyde solution at 4°C. The sections were then washed in four changes of double-distilled water. Slides were dipped in Ilford K5 emulsion (Ilford Ltd., Cheshire, U.K.) at 4°C in a darkroom and air-dried at room temperature before storage in a lightproof box for 4 days at 4°C. The autoradiograms were then developed and fixed. The sections were then washed in distilled water and stained for 4 min in Mayer's haematoxylin before mounting in Apathy's medium. Autoradiograms were viewed using an Olympus BH series light microscope and representative photomicrographs were recorded on 100 ASA colour reversal film (Kodak Ltd., Hertfordshire, U.K.). The binding studies were controlled by incubating sections with radioligands in the presence of a high molar excess (1 μM) of appropriate 'cold' peptide and specificity for each radioligand was determined by incubating radiolabelled VIP with 'cold' PHM and radiolabelled PHM with 'cold' VIP.

Collection and radioimmunoassay of human sweat

Exercise-induced sweat was collected from three athletes undergoing weight training. Each was asked to refrain from using underarm antiperspirants for 3 days and to shower immediately

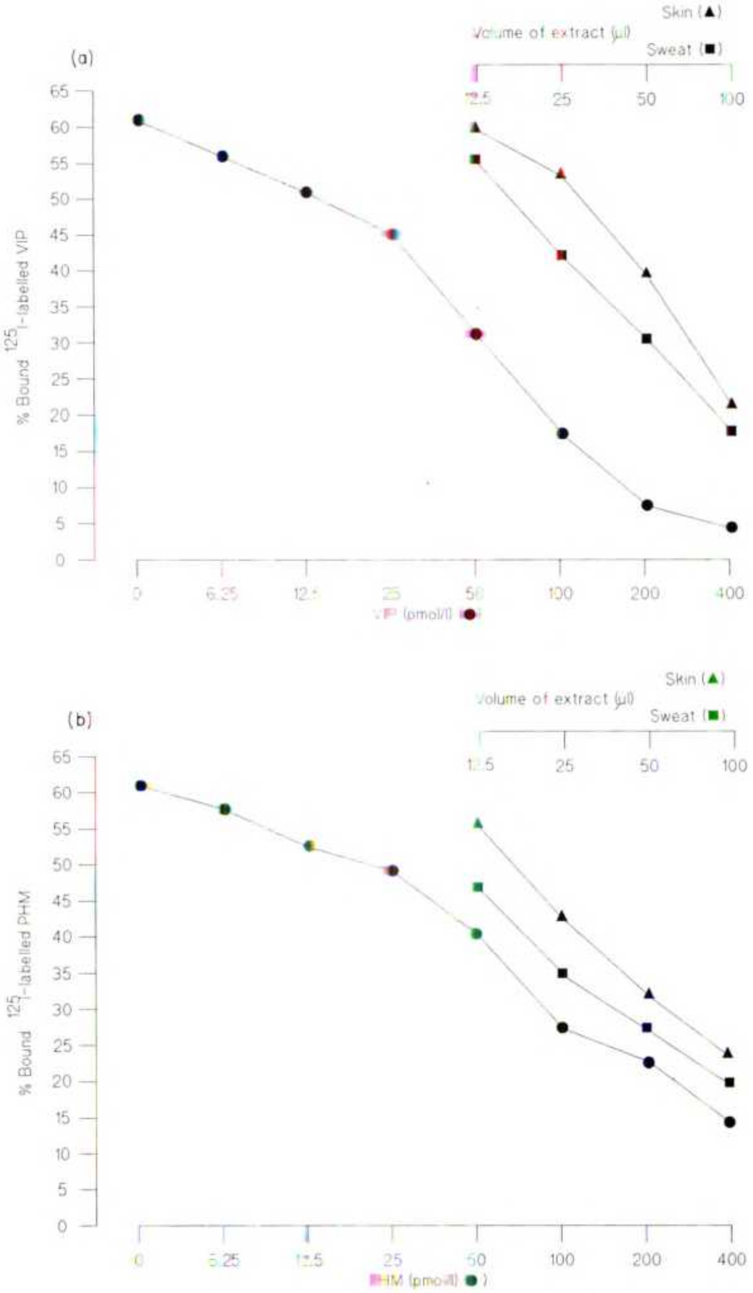

FIGURE 1. The effect of an axillary skin extract and a Sep-pak concentrate of sweat, on the binding of ^{125}I-labelled VIP (a) and ^{125}I-labelled PHM (b) to their appropriate antisera. The effect of increasing concentrations of synthetic peptides is indicated for comparison in each case.

before sweat collection using only water. Axillary sweat was collected during exercise into polypropylene tubes (Sarstedt, F.R.G.) containing 50 μl of a 0·4 mg/ml solution of Thiomerosal (BDH Chemicals Ltd) as a bacteriostat. Pooled sweat (12 ml) was centrifuged at 1000 ***g*** for 20 min to remove particulate skin debris, and following acidification with 1 ml of 1% (v/v) trifluoroacetic acid (TFA), peptides in the sample were concentrated and the sample desalted using a Sep-pak C-18 cartridge (Waters Associates, Milford, MA, U.S.A.). The cartridge was eluted with 3 ml of acetonitrile and the eluant was lyophilized. The sample was reconstituted in radiommunoassay buffer (see previous Methods section) and subjected to both VIP and PHM radioimmunoassays.

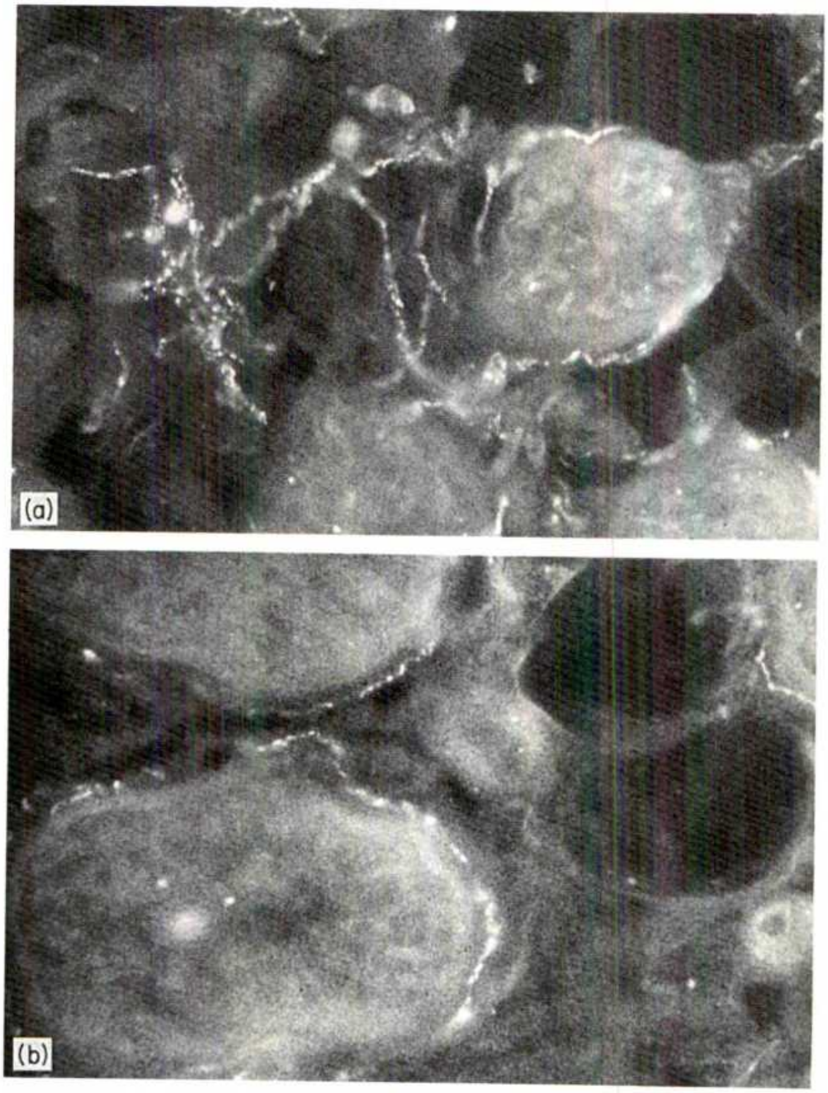

FIGURE 2. Immunofluorescence photomicrograph of VIP (a) and PHM (b) immunoreactive nerve fibres surrounding eccrine sweat glands in human axillary skin (× 400).

Reverse-phase HPLC

Dried extracts of axillary skin (2·4 g) and a lyophilized concentrate of an additional pooled sample of exercise-induced sweat (22 ml) were reconstituted in 2 ml of 0·1% (v/v) aqueous TFA and, after passage through a 0·22 μm filter, were injected in separate runs onto a Waters Z Module system fitted with a u-Bondapak C-18 cartridge, which was eluted with a linear gradient from TFA/water (0·1:99·9) to TFA/water/acetonitrile (0·1:39·9:60·0) in 60 min at a flow rate of 2 ml/min. Fractions of 2 ml were collected each minute and lyophilized prior to VIP and PHM radioimmunoassays. The column was subsequently calibrated with synthetic VIP and PHM whose retention times were determined spectrophotometrically.

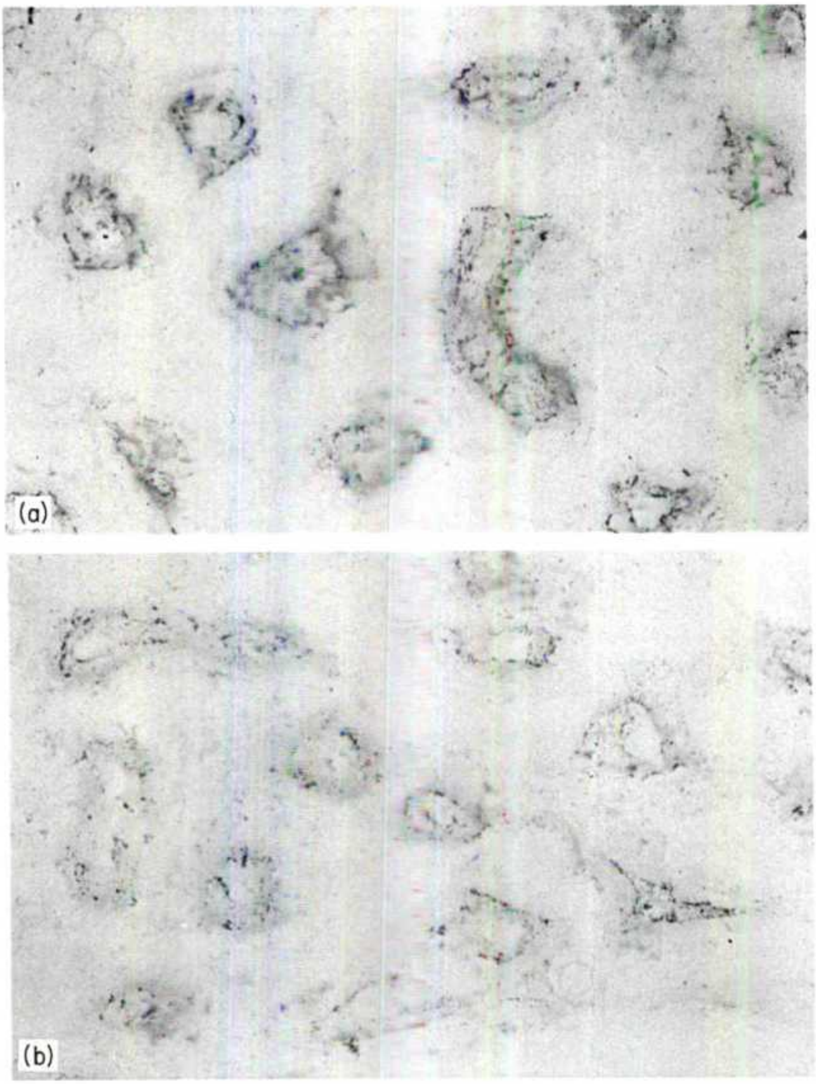

FIGURE 3. Autoradiogram showing ^{125}I-labelled VIP binding sites (a) and ^{125}I-labelled PHM binding sites (b) on eccrine gland secretory cells in human axillary skin (× 400).

RESULTS

Radioimmunoassay of axillary skin extracts

Each of the nine samples extracted contained VIP and PHM immunoreactivity. VIP immunoreactivity (7·63 + 2·33 pmol/g, x + SE) was consistently more abundant than PHM immunoreactivity (3·86 + 0·56 pmol/g, x + SE). Each immunoreactivity, when assayed in doubling dilutions, exhibited parallel displacement when compared with synthetic peptides (Figs 1a, b).

Immunocytochemistry

VIP. Dense networks of VIP immunoreactive nerve fibres were found in close association with eccrine sweat glands (Fig. 2a) and many fibres were in close contact with the membranes of glandular cells with others extending into the surrounding dermis. Only occasional fibres were detected in other skin structures, especially in the deep dermis, where they were associated with small blood vessels. No fibres were detected around either apocrine or sebaceous glands.

PHM. As expected, PHM immunoreactive fibres had a similar distribution to VIP

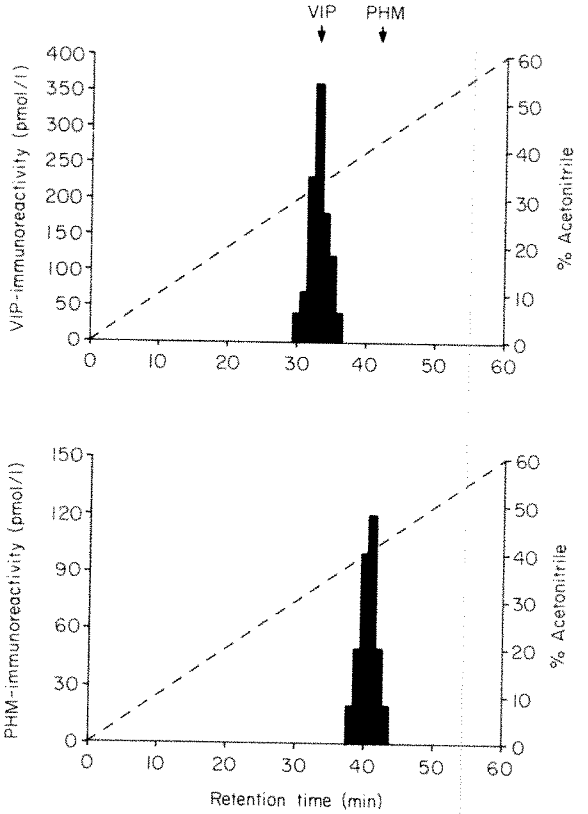

FIGURE 4. Reverse phase HPLC chromatogram of VIP immunoreactivity (upper panel) and PHM immunoreactivity (lower panel) in an extract of human axillary skin. The retention times of synthetic VIP and PHM are indicated.

immunoreactive fibres with a dense network of apparently finer calibre fibres around the eccrine sweat glands (Fig. 2b). These fibres, and those in the dermis, exhibited less immunofluorescence than those stained with the VIP antiserum.

Both immunocytochemical reactions were eliminated in control sections by the omission of a single layer of the immunocytochemical staining complex and staining was also abolished by pre-incubation of the primary antisera with as little as 100 ng/ml of appropriate peptide. However, pre-incubation of VIP antiserum with PHM (10 μg/ml) and PHM antiserum with VIP (10 μg/ml) had no discernible effect on immunostaining, indicating that each antiserum was specific for the peptide to which it was raised.

In vitro autoradiography

Grain formation, indicating the presence of radioiodinated VIP and PHM binding sites, was observed over the membranes of secretory cells within the eccrine sweat glands (Figs 3a, b). There was little non-specific grain formation and this was confined mainly to the dermis. No depositions were observed around apocrine glands. The presence of 1 μmol/l of 'cold' homologous peptide in the incubates almost completely abolished grain deposition over the eccrine sweat glands while no discernible effect was observed following addition of 'cold' PHM

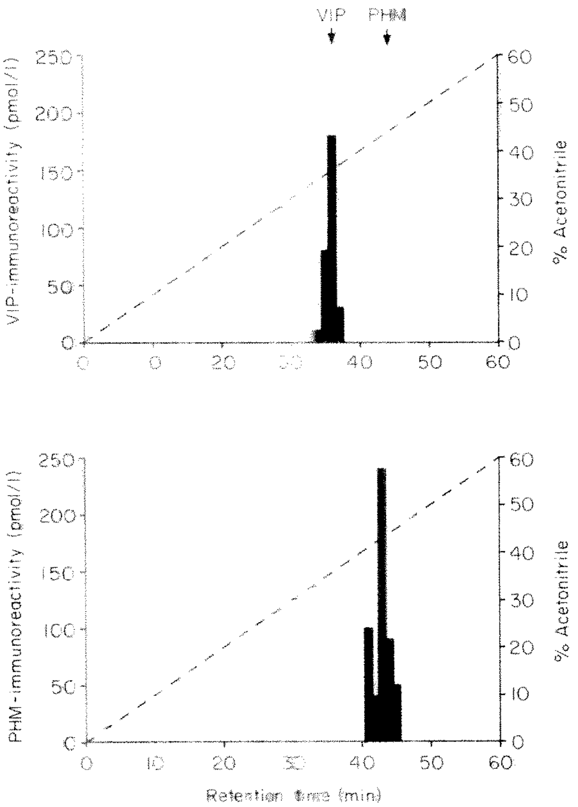

FIGURE 5. Reverse phase HPLC chromatogram of VIP immunoreactivity (upper panel) and PHM immunoreactivity (lower panel) in a Sep-pak concentrate of human sweat. The retention times of synthetic VIP and PHM are indicated.

to radioiodinated VIP and *vice versa*. These data indicate the presence of specific VIP and PHM binding sites on human eccrine sweat glands.

Radioimmunoassay of human sweat

Pooled human sweat contained both VIP immunoreactivity (30·6 pmol/l) and PHM immunoreactivity (43·4 pmol/l). Each peptide immunoreactivity exhibited parallel displacement in radioimmunoassay when compared with synthetic peptides and axillary skin extracts (Figs 1a, b).

Reverse-phase HPLC

VIP and PHM immunoreactivity in extracts of axillary skin eluted as single peaks, corresponding in retention time to synthetic VIP and PHM, respectively (Fig. 4). VIP immunoreactivity in sweat eluted as a single peak with an identical retention time to synthetic peptide, whereas PHM immunoreactivity eluted as two peaks. The latter major peak corresponded in retention time to synthetic standard. The earlier minor peak may represent PHM sulphoxide (Fig. 5).

DISCUSSION

VIP is a ubiquitous neuropeptide in mammals and is found in the nerves of both the central and peripheral nervous systems.[16] VIP immunoreactive nerve fibres have been demonstrated in the skin of several mammals including man,[17,18] where fibres and varicosities are present in close proximity to glandular cells, duct cells and myoepithelial cells. In the skin of the cat, the VIP immunoreactive fibres occurred in the same location around eccrine sweat glands as fibres containing acetylcholine, and following lumbar sympathectomy, both types of fibres disappeared, indicating a sympathetic origin.[17] In several studies,[9,10] VIP immunoreactive fibres have been identified around additional structures in the skin, including apocrine glands, hair follicles, sebaceous glands and immunoreactivity has been reported in the Merkel cells of the epidermis.[19] In the present study, using specimens of human axillary skin, VIP immunoreactive fibres were abundant around eccrine sweat glands and were not observed routinely in close association with any other structures, except for the occasional fibre in the deep dermis, apparently associated with small blood vessels. Although the distinct association of VIP immunoreactive fibres with eccrine sweat glands has been confirmed, the tentative relationship with other skin structures has not. The discrepancy in data may be attributable to such factors as differential antiserum specificity or to variability in the discrete microanatomical distribution of VIP immunoreactive fibres in the skin.

PHM,[2] the human analogue of porcine peptide histidine isoleucine (PHI),[20] was originally identified on the same precursor as VIP, and its structure was subsequently confirmed following its isolation from the colon.[21] As a consequence of co-translation, both peptides have an identical widespread distribution in mammalian tissues,[22,23] although in some tissues,[24] differential processing of precursor can give rise to extended molecular forms. There is a single report of PHM immunoreactive fibres in human skin.[10] Smooth fine calibre fibres were identified around eccrine sweat glands, apocrine glands, hair follicles, blood vessels and sebaceous glands. As was the case for VIP immunoreactive fibre distribution in the present study, PHM immunoreactive fibres were localized predominantly around eccrine sweat glands with a few fibres around small vessels in the deep dermis. Although co-localization studies were not performed, the distribution of both types of fibres in this study were identical. This, we consider, increases the validity of the distribution patterns described and substantiates previous assertions that both peptides are co-localized in the same neurones. However, the reasons cited previously for the discrepancy in VIP fibre distribution may still apply. When using immunocytochemical antisera to peptides such as VIP and PHM, which exhibit a high degree of primary structural homology, it is essential to control for the possibility of cross-reaction. As pre-adsorption of each antiserum was completely effected by addition of 100 ng/ml of homologous peptide but immunostaining intensity was unaffected by pre-incubation with a 100-fold greater concentration of heterologous peptide, we feel confident in the assertion of antiserum specificity.

Autoradiography of suitably stabilized sections of axillary skin, using radioiodinated VIP and PHM, identified specific binding sites for each peptide on the membranes of eccrine gland secretory cells. These sites were in close proximity to the peptidergic fibres previously described, inferring that the eccrine gland secretions are in part controlled by these peptides. Several recent studies have postulated a role for VIP in the production of sweat by direct action on the eccrine gland. Using isolated monkey eccrine sweat glands derived from palmar skin, Sato and Sato[25] have demonstrated that concentrations of VIP as low as 1 nM, stimulated the secretion of sweat in a time course which paralleled cAMP accumulation in the secretory cells. They noted that sweating induced by VIP was as profuse as that induced by isoproterenol. Sweating could be achieved by VIP at two orders of magnitude lower than isoproterenol, but this effect was short-lived, presumably due to desensitization of receptors or degradation of peptide. VIP, when applied to a primary culture of avian salt gland cells, stimulated active ion transport in a dose-dependent fashion which was synergistic with the effect of acetylcholine.[26] Specific high-affinity VIP receptors have been identified on isolated human eccrine sweat glands[27] which have low affinity for porcine PHI and which are linked to membrane-bound adenylate cyclase.[28] These reports substantiate the distribution of VIP and PHM binding sites and peptidergic innervation described in this study, which are in keeping with the findings of Tainio,[28] who failed to demonstrate binding sites on apocrine glands, blood vessels or dermal fibroblasts. VIP, and presumably PHM, are therefore strategically placed to participate in the direct stimulation of human eccrine sweat rather than, as has been previously suggested, to facilitate vasodilation of blood vessels supplying the glands.[29] This effect may be synergistic with acetylcholine which, in the cat,[17] and human,[30] is co-localized with VIP in postganglionic sympathetic neurones innervating the sweat glands. The putative central role for these neuropeptides in the production of human sweat may aid the understanding of the mechanisms by which aberrations in electrolyte secretion occur in conditions such as cystic fibrosis, and indeed, in individuals suffering from this condition, a decrease in the numbers of VIP immunoreactive fibres innervating the eccrine sweat glands has been observed.[31]

VIP and PHM have been detected by radioimmunoassay in extracts of human axillary skin, a site which contained the highest levels of VIP in a previous distribution study of human skin.[18] The levels of VIP immunoreactivity were consistently higher than PHM immunoreactivity. This observation may simply reflect the different chemical properties of each peptide, resulting in a lower recovery of PHM, or may indicate the presence of the C-terminally extended form of PHM, designated PHV (peptide histidine valine),[24] in cutaneous peptidergic neurones. This molecular form is not detectable with the C-terminally directed antiserum used in this study. The discrepancy in levels of VIP and PHM immunoreactivity in extracts of human skin has been previously reported.[32] Reverse-phase HPLC analysis of peptide immunoreactivity in skin extracts identified two resolved peptides, co-eluting with synthetic VIP and PHM respectively, indicating that the levels of peptide immunoreactivity determined in crude skin extracts were not artefactual as can often be the case when one measures small amounts of peptides in protein-rich solutions.

A rather unexpected finding in this study was the presence of both VIP and PHM immunoreactivity in sweat. As high concentrations of salts in radioimmunoassay samples can often likewise lead to spurious artefactual results, all sweat samples were desalted prior to radioimmunometric analysis. The levels of PHM immunoreactivity in such samples were consistently higher than those of VIP, which may reflect the greater recovery of the former, more hydrophobic peptide, or indicate its greater stability in the eccrine secretions. Reverse phase HPLC analysis confirmed the chromatographic identity of each immunoreactive peptide although a small amount of the PHM was apparently oxidized, a phenomenon often encountered with peptides containing methionine residues. The function of these peptides in the secretions of the eccrine sweat glands is unclear, but their occurrence most probably represents a 'washout' phenomenon as has been noted for VIP and acetylcholine in the venous effluent of the cat submandibular gland following lingual nerve stimulation,[33] or for acetylcholine in the venous effluent of cat paws which accompanies sweating induced by stimulation of the sympathetic chain.[34]

The presence of VIP and PHM immunoreactive fibres and their corresponding specific binding sites around human eccrine sweat glands, coupled with their presence in secretions, provides strong evidence for their involvement in the physiological regulation of eccrine sweating in man. It appears that they may act synergistically with the classical transmitter, acetylcholine, by a mechanism which requires further study.

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Measurement and perception of skin colour in a skin cancer survey

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SUMMARY

A population-based prevalence survey of skin cancer was conducted in Nambour, Queensland, in 1986. The skin colour of 807 participants was assessed in three ways: quantitatively, graded by a dermatologist, and self-reported. Quantitation of skin pigmentation was obtained by measuring the reflectance of light of wavelength 650 nm, at six sites. Females showed higher mean reflectance (paler skin) than males at all sites with the greatest difference on the lateral forearms. Prevalent skin cancer in males, and solar keratoses in both sexes were correlated with inherently pale skin colour on an unexposed site, and the presence of keratoses was correlated with darkly-pigmented backs of the hands ($P < 0.001$). Both dermatologists' and participants' grading of skin colour were moderately correlated with measured skin colour. For dermatologists, correlation was highest with reflectance from the medial upper arms ($r = 0.35$, right arm; 0.30, left) in males, and the lateral forearms ($r = 0.34$, right; 0.38, left) in females. Correlations between reflectance values and self-reported innate skin colour were highest for the upper arms ($r = 0.26$, right; 0.24, left) in males, and for forearms ($r = 0.42$, right and left) in females. Prevalence of actinic lesions was more highly correlated with subjectively assessed skin colour than with quantitative skin pigmentation.

Skin colour is a major determinant of melanoma and non-melanoma skin cancer, yet the problem of accurately measuring skin colour has rarely been considered in studies of skin malignancy. Modern investigators[1–5] tend to rely upon a subjective grading of skin pigmentation e.g., fair, medium, olive or black, although the validity and reliability of judging skin colour are largely unknown.

Edwards and Duntley[6] first quantified skin colour 50 years ago by measuring visible light reflectance of the skin with a spectrophotometer. While reflectance measures have been used subsequently in a range of anthropological and genetic studies,[7–11] few epidemiological studies of skin cancer have used objective means of estimating degree of pigmentation. Although in two case-control studies of cutaneous melanoma[12,13] investigators estimated skin reflectance, final

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values were obtained indirectly, so evaluation was still partly subjective. In a study of melanoma in British Columbia, observer perception of skin colour was assessed.[13] Six observers each evaluated the skin colour of eight subjects under three lighting conditions (daylight, fluorescent and incandescent light sources), and skin tones were transformed to reflectance values measured at four wavelengths. At 400–600 nm, approximately 30% of the variance was due to observers, light source, time of observation and error, but these factors accounted for 75% of the variance at wavelengths of 700 nm.[13]

During a community survey of skin cancer we measured the skin colour of 807 subjects by reflectance spectrophotometry. Also, dermatologists rated skin colour on a four-point scale, and subjects rated their own skin colour on the same scale. In this paper the relation between quantitative skin colour and prevalence of skin cancer is investigated, and objective skin-colour measurement is compared with the subjective assessment of skin colour by dermatologists, and with self-reported skin colour.

METHODS

A population-based survey of skin cancer was conducted in Nambour, Queensland, in December, 1986. Full details have been reported previously.[14] A random sample of 3000 was chosen from the 5100 residents aged 20–69 years who were enrolled in the Nambour state electorate (voter registration is compulsory in Australia). A total of 2095 people took part, with a response rate among permanent residents of 78%. Information obtained during a standard interview included: patterns of occupational and recreational sun exposure which participants classified overall as 'mainly outdoor', 'both indoor and outdoor' or 'mainly indoor' (variable labels, OCCEXP and RECEXP); acute reaction of untanned skin to strong sun, classified as 'always burn, never tan', 'burn then tan' or 'only tan' (BURNTAN); number of painful sunburns in life (SUNBURN); and past history of skin cancer (PREVSKCA). Each person was also asked, 'How would you rate your natural skin colour on areas never exposed to the sun (like under your arm): fair, medium, olive or black?' (SKCLSELF). Response categories for all items are shown in Table 1.

Every subject was examined by one of 14 dermatologists. In 90% of subjects, only the head and neck, backs of hands and forearms were inspected, while a random 10% of subjects received a full skin examination. The dermatologists assessed natural skin colour (SKCLDERM), eye and hair colour (EYECLR, HAIRCLR); as well as number of solar keratoses (CLINSK) and skin cancers on all subjects. Any basal cell carcinoma or squamous cell carcinoma diagnosed clinically was biopsied, and confirmed by histology to be invasive skin cancer (HISTSCA).

Measurements were made of the reflectance of light of wavelength 650 nm from the skins of all subjects who attended during the first 2 days of the survey, a random sample of survey participants. An EEL Model 99 Reflectance Spectrophotometer (manufactured by Diffusion Systems Ltd, Hanwell, London) was used.[15] Reflectance at 650 nm is not affected by blood or carotene pigments in the skin and depends mainly on the amount of melanin pigment present, and to some extent on the thickness of the overlying skin.[7] The instrument was standardized initially and between subjects, so that reflectance values were relative to 100% reflectance from a standard white tile which itself had 85% reflectance of the absolute standard of a magnesium carbonate surface. Thus, high reflectance values indicate pale skin with little absorptive melanin pigment. Readings were made by one of two operators at six sites on each subject, namely the medial aspects of the right and left upper arms midway between axilla and elbow (UPPARMR, UPPARML); at the lateral aspects of both forearms midway between elbow and wrist (FORARMR, FORARML); and on the backs of both hands (HANDR, HANDL).

TABLE 1. Distribution of skin cancer and risk factors by sex in 346 males and 461 females in Nambour, Queensland, 1986

Occupational exposure	Outdoor	In/Out	Indoor			
Males (%)	41·0	35·5	23·5			
Females (%)	9·3	31·5	59·2			
Leisure exposure	Outdoor	In/Out	Indoor			
Males (%)	64·5	26·3	9·2			
Females (%)	38·8	37·7	23·5			
Sunburn	Never	Once	2–5 ×	> 5 ×		
Males (%)	12·1	12·4	43·4	32·1		
Females (%)	12·8	15·6	47·9	23·7		
Previous skin cancer	No	Yes				
Males (%)	79·9	20·1				
Females (%)	81·2	18·8				
Burn or Tan	Burn	Burn/Tan	Tan			
Males (%)	18·5	65·0	16·5			
Females (%)	29·1	56·6	14·3			
Skin colour (self)	Fair	Medium	Olive	Black		
Males (%)	60·1	31·5	8·1	0·3		
Females (%)	63·6	28·0	8·4			
Skin colour (doctor)	Fair	Medium	Olive	Black		
Males (%)	40·6	45·8	12·8	0·9		
Females (%)	47·0	42·0	10·7	0·3		
Eye colour (doctor)	Blue/ Grey	Light brown	Green hazel	Dark brown		
Males (%)	49·3	33·3	6·7	10·7		
Females (%)	41·4	38·3	7·0	13·3		
Hair colour, age 21 (doctor)	Blonde	Light brown	Ginger	Dark red	Dark brown	Black
Males (%)	8·4	35·1	3·2	1·7	42·6	9·0
Females (%)	8·0	39·6	5·0	4·3	37·6	5·4
Solar keratoses (doctor)	No	Yes				
Males (%)	49·7	50·3				
Females (%)	62·3	37·7				
Skin cancer (histology)	No	Yes				
Males (%)	96·8	3·2				
Females (%)	98·7	1·3				

All variables are coded in ascending order of categories as shown.

Analysis

Correlations between variables were investigated, as the primary concerns were to compare objective measurement of skin colour to subjective assessment and to evaluate the relation between objective measurement and standard outcomes and risk factors related to skin cancer. For ordinal variables, correlations were calculated assuming that there is a normal distribution of liability underlying the categories. PRELIS 1.8[16] was used to calculate separate correlation matrices for males and females, with pairwise deletion of missing values. Between two continuous traits, Pearson correlations were calculated; between two ordinal variables polychoric correlations were used;[17] and a polyserial correlation was calculated between a continuous and an ordinal variable.[18]

RESULTS

Distribution of reflectance measurements

Reflectance measurements of skin colour were performed on 807 subjects, 346 males and 461 females (average age 44 years). Females showed higher mean reflectance than males at all sites. The difference between sexes was greatest for the lateral forearms and least for the medial surface of the upper arms, where the highest readings were obtained in both sexes (Table 2). The forearms had higher reflectance values than the hands in females, while the hands of males had the higher values. Laterality had little influence with the exception that the right forearm tended to be darker than the left in both sexes.

Inter-observer reliability of reflectance measurements

Two observers made all the reflectance measurements. Inter-observer reliabilities were calculated as Pearson correlations between independent reflectance measurements taken by both observers in 43 subjects at all six sites (Table 2). All were highly significant and large, though the magnitude of reliability at a given site was directly proportional to the variance of

TABLE 2. Distribution and inter-observer reliability of skin reflectance measurements and age in 346 males and 461 females in Nambour, Queensland, 1986

		Mean	SD	Skewness	Range	Reliability*
Age	Male	44·3	14·2	0·069	20–69	
	Female	44·4	13·1	0·059	20–69	
Upper arm						
Left	Male	74·4	4·6	−0·982	48–86	0·79
	Female	77·2	3·6	−0·510	60–85	
Right	Male	74·1	4·5	−0·903	49–85	0·79
	Female	76·8	3·6	−0·800	55–86	
Forearm						
Left	Male	50·4	7·8	−0·168	28–69	0·96
	Female	65·9	5·1	−0·605	43–81	
Right	Male	47·3	8·2	0·196	23–73	0·95
	Female	63·7	5·5	−0·899	35–76	
Hand						
Left	Male	55·7	6·7	−0·506	30–75	0·92
	Female	61·7	6·3	−0·193	40–80	
Right	Male	55·1	6·5	−0·229	34–72	0·89
	Female	62·0	6·2	−0·459	31–80	

* Pearson correlation of measurements by two observers of 43 subjects.

reflectance measurements at that site. Thus, reliability of measurement was greatest for the forearm (0·95) and least for upper arm (0·79), where variance in skin colour was only about 30% of that on the forearm (Table 2). Measurement error therefore formed a greater proportion of the total variance of reflectance on the upper arm.

Prevalence of risk factors and outcomes

Conventional sun-related risk factors for skin cancer were predominant in males (Table 1), and these results have been discussed in detail elsewhere.[14] Dermatologists evaluating skin colour rated the majority (46%) of men as having medium complexions and the majority (47%) of women as having fair skin. By contrast, 60% of men and 64% of women considered themselves to have fair skin. Half of all the males examined had solar keratoses, and 3% had skin cancer. Fewer females were affected and 38% had keratoses and 1% skin cancer (Table 1).

Correlation between skin colour and outcomes

Despite the small number of skin cancers diagnosed during the survey, there were significant correlations ($P < 0·001$) with several reflectance measurements in both males and females, though actual patterns of association were variable (Table 3). The presence of skin cancer diagnosed in 11 males was correlated with high reflectance values (pale skin) on the medial aspect of the upper arms, but was not related to measured pigment of forearms or backs of hands. Skin cancer diagnosed in five females was correlated with low reflectance values on the medial upper arms and on the forearms, with no association with colour of backs of hands. Solar keratoses showed quite different associations (Table 3). Among males (174 of whom were affected), keratoses were correlated with high reflectance values of the medial upper arm, and with low values on the lateral forearms and hands. Keratoses in females (174 affected) were associated ($P < 0·001$) only with low reflectance (dark pigmentation) on the backs of the hands.

Prevalence of skin cancer and self-graded fair skin colour in males were highly correlated—greater than skin colour as assessed by the dermatologist or objective measurement of pigmentation. Correlations between prevalence of solar keratoses among males and both self-reported fair skin colour and measured pale pigmentation on medial upper arms were similar, and the highest correlations were with darkly pigmented forearms and backs of hands. There were moderate negative correlations between high reflectance from the upper arms (pale skin) and prevalence of skin cancer and of solar keratoses in females, but the reverse with skin colour as perceived by dermatologists and self.

Correlations of skin colour with other risk factors

High reflectance of the upper arms was associated with increasing age in males but not in females. Low reflectance from forearms and hands (dark pigmentation) was associated with increasing age in both sexes ($P < 0·001$) (Table 3). Other risk factors for skin cancer which were significantly correlated with low reflectance values on forearms and hands among males and females (though site-specific values varied) were outdoor occupations and leisure activities. High reflectance values on upper arms were associated with number of episodes of severe sunburn, and in males, previous skin cancer; propensity to burn rather than tan; and hair colour. Eye colour was associated with reflectance values in males, but showed no association in females (Table 3).

Correlations between subjective and objective measurements of skin colour

Significant correlations were observed between subjective rating (by dermatologists and self) of

TABLE 3. Correlations (× 100) of skin reflectance measurements with outcome variables and other risk factors in the Nambour Skin Cancer Survey, 1986

Males ($n = 345$–346)

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
1 AGE	100	−08	−27	21	45	−10	−08	−03	02	−19	73	49	20	26	−31	−32	−43	−35
2 OCCEXP	−17	100	20	15	−14	−11	−20	11	03	05	−19	−07	13	07	23	22	28	26
3 LESEXP	−07	14	100	03	−25	−02	−11	13	07	03	−30	−08	−02	−01	24	23	27	16
4 SUNBURN	−34	03	−03	100	02	−20	−18	−10	01	−09	−03	03	05	05	13	05	18	16
5 PREVSKCA	35	−07	−09	−02	100	−20	−10	−13	−16	−26	54	51	25	24	−12	−17	−16	−04
6 SKCLSELF	03	02	−09	−15	−18	100	53	43	36	65	−26	−91	−24	−26	−21	21	−13	−23
7 BURNTAN	10	−19	−16	−32	−07	55	100	11	14	33	−25	−13	−22	−20	−05	−10	−14	−20
8 EYECLR	12	01	−02	−12	−16	31	08	100	42	52	−10	−08	−23	−20	−26	−27	−08	−16
9 HAIRCLR	01	−09	−06	−16	−01	27	17	34	100	55	−02	−21	−23	−16	−30	−35	−14	−23
10 SKCLDERM	−04	−09	−12	−22	−20	60	49	30	45	100	−48	−60	−30	−33	−31	−20	−12	−19
11 CLINSK	53	−15	−04	−10	37	−30	−07	−17	−15	−39	100	93	20	22	32	−28	−39	−18
12 HISTSCA	10	−32	−10	−04	−01	−25	11	15	14	−11	28	100	33	30	04	02	02	06
13 UPPARML	−04	−01	03	03	03	−29	−21	−05	−07	−20	−07	−20	100	71	25	26	18	22
14 UPPARMR	01	06	10	04	03	−37	−31	−09	−11	−28	13	−18	74	100	18	16	14	18
15 FORARML	−17	18	19	10	−11	−42	−43	−10	−20	−38	01	−21	39	45	100	85	52	59
16 FORARMR	−18	14	25	11	−01	−42	−44	−09	−23	−34	02	−19	36	40	79	100	49	58
17 HANDL	−50	14	09	28	−17	−35	−31	00	−02	−22	−16	07	28	31	60	52	100	80
18 HANDR	−49	15	14	28	−18	−31	−29	01	−06	−22	−17	−06	33	32	59	58	83	100

Females ($n = 457$–461)

For males (above diagonal), $P < 0{\cdot}05$ for $|r| > 0{\cdot}11$, $P < 0{\cdot}001$ for $|r| > 0{\cdot}17$. Corresponding values for females (below diagonal) are 0·09 and 0·15. Intercorrelations of continuous variables (1, 13–18) are Pearson, of ordinal variables (2–12) are polychoric, and between continuous and ordinal variables are polyserial.

fair skin and objective measurement of high reflectance at all sites, and correlations were highest for the upper arms in males, and the forearms in females (Table 3). Compared to dermatologists' assessments, self-reporting of skin colour by females was more highly correlated with the reflectance values, whereas that by males was less or similarly correlated.

There are 306 correlations listed in Table 3 so some of them will be significant by chance. Nevertheless, the 95% confidence intervals are around ±0·10 so the point estimates give a fair idea of the importance of relationships between variables. In many cases, confidence in a correlation is enhanced if it is replicated in both sexes, although there may be a genuine sex difference.

DISCUSSION

These quantitative measurements of skin colour in an unselected population confirm that exposed skin surfaces are considerably darker in males than females, while the colour of skin not exposed to the sun is slightly darker in males (Table 2). Sexual dimorphism in inherent skin colour has long been observed,[19] and may be related to differential levels of melanocyte stimulating hormones.[20] That men in general spend much more time outdoors than women (Table 1) suggests that tanning (facultative pigmentation), and perhaps skin thickening in response to sun exposure, are responsible for the sex variation in colour of exposed skin. Another factor contributed to the lower reflectance values in males, namely dark hair which occurs more commonly on the arms. For this reason the forearms, which are less sun-exposed than the backs of the hands overall (evidenced by lighter colour of forearms than backs of hands in females), showed deeper pigmentation than the hands in males. The darker colour of the right forearm compared to the left in both sexes is presumably due to sun exposure while driving.

Light innate skin colour was significantly associated with skin cancer in both males and females. In the females the pale forearms were also associated with skin cancer. Keratoses occurred in both males and females with a naturally light skin, but whose hands tended to be darkly tanned. Inconsistencies in patterns of association between skin cancers and keratoses may have been partly due to the small number of subjects with prevalent skin cancer, or they could signify different aetiologies of basal cell carcinoma (the predominant type of skin cancer) and solar keratoses.

These findings agree with the finding that actinic lesions are most frequently seen in fair skinned people who are repeatedly outdoors. On this basis, the level of correlation between cancers and keratoses on the skin and objective skin colour measurements, compared to qualitative skin colour (graded by an expert or self), were lower than expected. The low correlations with reflectance measures may indicate that it is not skin colour *per se*, but some combination of factors that determines the development of skin tumours. Many of these factors relate to sun-sensitivity of the skin, e.g. propensity to sunburn, and are thus significantly correlated with skin colour (Table 3), and with the ability to tan.

Neither the examining dermatologists nor the subjects themselves accurately graded skin colour (Table 3). As subjects and dermatologists were asked to rate untanned skin colour, the correlations of interest are between upper arm measurements and ratings, the highest of which ($r = -0{\cdot}37$) was between self-rating and right upper arm reflectance in females. By this criterion, female subjects rated their own skin colour slightly more accurately than did dermatologists, whereas in males the reverse was true. However, somewhat higher correlations between tanned skin measurements on forearms and both self and doctor ratings were seen in females, suggesting that their perception of skin colour was more influenced by tanned than untanned skin colour, in spite of contrary instructions (this effect was not seen in males). Variability of pigmentation between males and females with advancing age as shown here, has also been observed in Indian women by Banerjee.[20] The reason for the lack of correlation between skin reflectance and eye colour among females in our study is unknown.

In conclusion, our study confirms the importance of skin colour of unexposed and sun-exposed sites, in relation to skin cancer. While the human eye is unable to estimate pigmentation precisely, it would seem that 'skin colour' ratings by dermatologists, and as perceived by self, are useful composite indicators of skin cancer susceptibility.

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Evaluation of public education campaigns in cutaneous melanoma: the King's College Hospital experience

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SUMMARY

The effects of a public education campaign directed at earlier self-recognition of malignant melanoma were assessed at King's College Hospital by reviewing the pathology of all melanomas seen between 1970 and 1987. During the campaign years (1986 and 1987) the number of patients with melanomas nearly doubled, and there was an increase in the proportion of thin, good prognosis lesions, and a fall in the proportion and absolute number of thick lesions. Data from the earlier years showed that median melanoma thickness had been decreasing throughout the series, well before the introduction of the campaign. A greater proportion of tumours of the superficial spreading variety was seen and also an increase in patients below the age of 40. These findings suggest that the biological characteristics of melanomas might be changing in addition to increased public and professional awareness.

Malignant melanoma has reached epidemic proportions in the U.K., and its incidence[1] and mortality[2] continue to rise. The depth of invasion (or Breslow thickness[3]) has been shown to be the most useful guide to prognosis. The early diagnosis at a thinner and curable stage has therefore received much attention. A number of reports[4–6] have described the effects of public education campaigns in potentially improving the prognosis of cutaneous malignant melanoma, by increasing the proportion of thin and decreasing the proportion of thick lesions that are seen.

Following the work of Doherty and MacKie[7] which demonstrated the need for public education in the recognition of melanoma at an earlier and curable stage, seven Cancer Research Campaign-funded centres were set up in the U.K. with the intention of assessing the effects of nationwide publicity campaigns aimed at earlier self-recognition of melanoma. King's College Hospital was designated such a centre, and a publicity campaign which included local press, radio and television releases was launched in 1986. In order to assess whether our campaign produced a similar improvement in early diagnosis as in other centres, it was decided to obtain

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baseline data by reviewing the pathology of cutaneous melanoma seen over the last 18 years at King's College Hospital.

METHODS

All pathological material reported as malignant melanoma at King's College Hospital from 1970 to 1987 was reviewed for diagnosis, tumour thickness as defined by Breslow[3] and growth pattern as defined by Clark *et al.*[8]

The identity of the patients was concealed to avoid any bias in measurement. With cases prior to 1979, when measurement of maximum tumour thickness was not performed routinely, the paraffin blocks that were not cut centrally were resectioned to minimize an under-representation of maximum tumour thickness. If the orientation of the specimen was in question upon inspection of the original slides, the blocks were also resectioned to minimize an overestimation of tumour thickness. Melanin bleach and Fontana silver stains were performed where needed. All patients were White with the exception of one Chinese female.

The campaign at King's College Hospital began by preparing local doctors with illustrated booklets, posters and lectures relating to melanoma. Following this, several articles appeared in

TABLE 1. Melanoma thickness, histological growth pattern and year of presentation (percentages rounded off to nearest 0·5%, are shown in brackets)

Year	Number melanomas	Median thickness (mm)	Thickness			Histological growth pattern	
			Thin	Intermediate	Thick	Superficial spreading	Nodular
1970	3	5·27	0 (0)	0 (0)	3 (100)	0 (0)	3 (100)
1971	8	1·78	3 (37·5)	2 (25)	3 (37·5)	2 (25)	5 (62·5)
1972	11	2·90	3 (27·5)	3 (27·5)	5 (45)	5 (45·5)	6 (54·5)
1973	4	3·50	0 (0)	2 (50)	2 (50)	0 (0)	4 (100)
1974	11	3·10	4 (36·5)	2 (18·5)	5 (45)	4 (36·5)	7 (63·5)
1975	6	5·40	0 (0)	(17)	5 (83)	0 (0)	6 (100)
1976	9	3·82	3 (33)	1 (11)	5 (56)	3 (33·5)	6 (66·5)
1977	5	4·18	0 (0)	2 (40)	3 (60)	1 (20)	4 (80)
1978	9	0·54	5 (55·5)	1 (11)	3 (33·5)	4 (44·5)	4 (44·5)
1979	8	2·31	3 (37·5)	4 (50)	1 (12·5)	3 (37·5)	3 (37·5)
1980	6	4·82	1 (17)	2 (33)	3 (50)	1 (16·5)	4 (66·5)
1981	4	0·44	4 (100)	0 (0)	0 (0)	4 (100)	0 (0)
1982	10	1·35	6 (60)	0 (0)	4 (40)	2 (20)	8 (80)
1983	17	1·80	8 (47)	5 (29·5)	4 (23·5)	9 (53)	7 (41)
1984	9	0·90	7 (78)	1 (11)	1 (11)	4 (44·5)	4 (44·5)
1985	10	1·17	6 (60)	2 (20)	2 (20)	6 (60)	2 (20)
1986	22	0·75	17 (77)	4 (18)	1 (5)	17 (77)	4 (18)
1987	24	1·05	15 (62·5)	7 (29)	2 (8·5)	14 (58)	9 (37·5)
Total	176	1·64	85 (48)	39 (22)	52 (30)	79 (45)	86 (49)

The percentages of superficial spreading and nodular melanoma do not add up to 100% as lentigo maligna melanoma and acral lentiginous melanoma are not shown above.

the local press, and there was national radio and television coverage on the topic during the late spring of 1986 and 1987. Patients were referred by their general practitioners to a pigmented lesion clinic held weekly at King's College Hospital.

RESULTS

Two-hundred and twenty-nine lesions were reviewed. Changes were confined to the epidermis (melanoma-*in-situ*) in 17 cases and these were excluded to allow comparison with other studies. Other categories that were excluded comprised eight patients who were stage II or more at presentation, seven metastatic deposits and four mucosal lesions. The diagnosis was revised in 17 cases from malignant to benign melanocytic lesions, mainly dysplastic naevus.[9] This left a total of 176 stage I cutaneous melanomas, which comprised 86 nodular melanomas, 79 superficial spreading melanomas, nine lentigo maligna melanomas and two acral lentiginous melanomas, the features of which are summarized in Table 1.

Tumour thickness

A change in the distribution of tumour thickness was seen throughout the 18-year series (Fig. 1), a trend which changed very little during the years of the campaign. The greatest changes in tumour thickness were seen in the late 1970s when median melanoma thickness fell from approximately 4 to 1·5 mm. The overall trend for an increase in the proportion of thin lesions (< 1·5 mm) was mirrored by a decrease in the proportion (and absolute number) of thicker lesions (> 3·5 mm) with the proportion of intermediate thickness lesions (1·5 to 3·49 mm) remaining roughly the same. This trend was also seen when each melanoma thickness was plotted against month of presentation over the 18 year period (Fig. 2a). This is shown in the scattergram (Fig. 2b) of the last 6 years (1981–1987) which suggests a clustering of thin tumours in the summer and autumn of 1987, although a similar but less marked seasonal variation of thin tumours was present in 1984, 1985 and 1986.

Growth pattern

An increase in the proportion of superficial spreading melanomas (Fig. 3) and a decrease in the proportion of nodular melanomas occurred throughout the 18-year period with very little

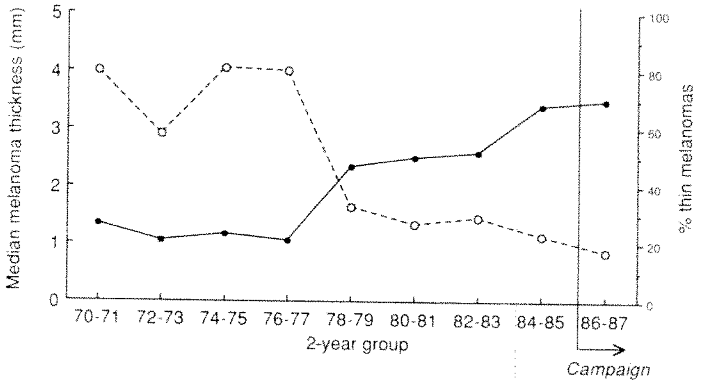

FIGURE 1. Median melanoma thickness and percentage of thin lesions (< 1·5 mm) and period of presentation. ○– – –○, median melanoma thickness; ●——● percentage thin melanomas.

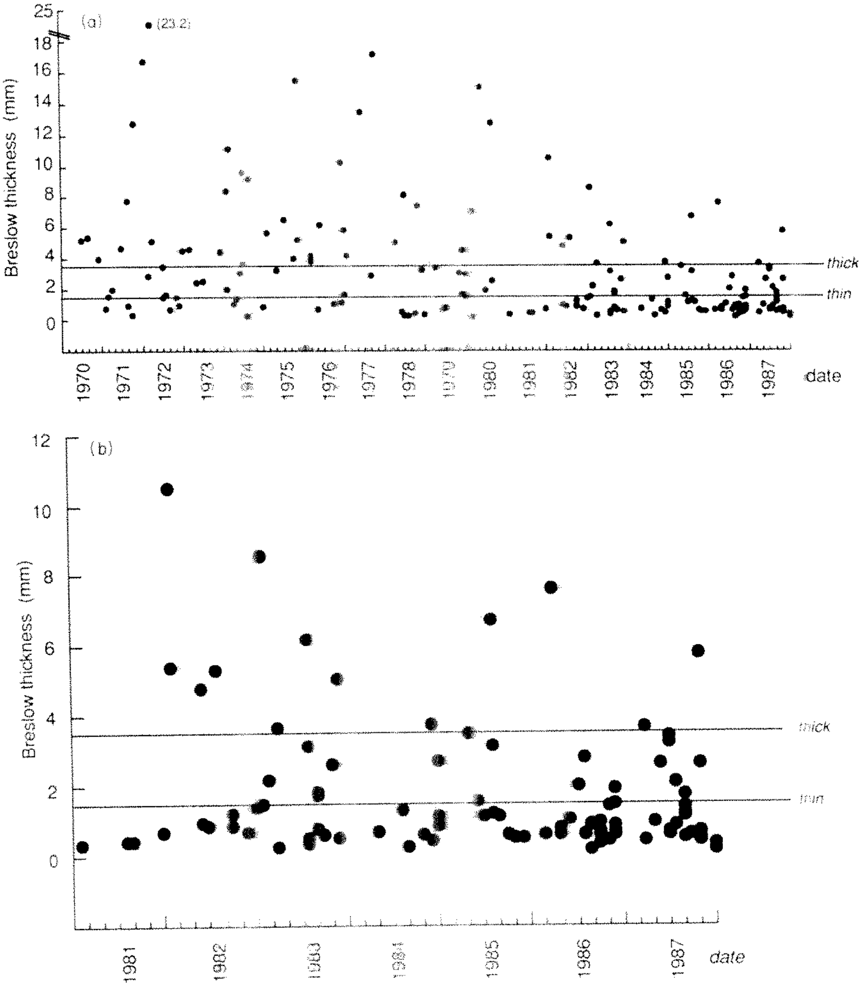

FIGURE 2. (a) Scattergram of Breslow thickness against month of presentation of 176 stage I melanomas during 1970–1987 (minor date divisions represent 2-monthly periods). (b) Scattergram of thickness against month of presentation for each stage I melanoma between 1981 and 1987 (minor date divisions represent 2-monthly periods).

alteration during the campaign years. Seven out of nine of the lentigo maligna melanoma lesions were seen in the latter half of the 18 year series, as were both acral lentiginous melanomas.

Age at presentation

The proportion of patients below the age of 40 presenting with Stage I melanoma increased throughout the series (Fig. 4), although other age groups did not show such a trend.

Site of primary and sex of patient

Neither of these variables demonstrated any clear trends within the 18-year study period.

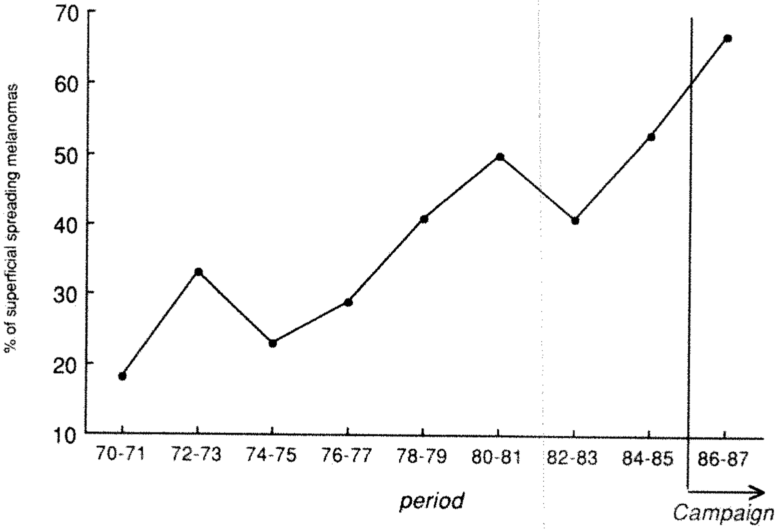

FIGURE 3. Proportion of superficial spreading melanomas and year of presentation.

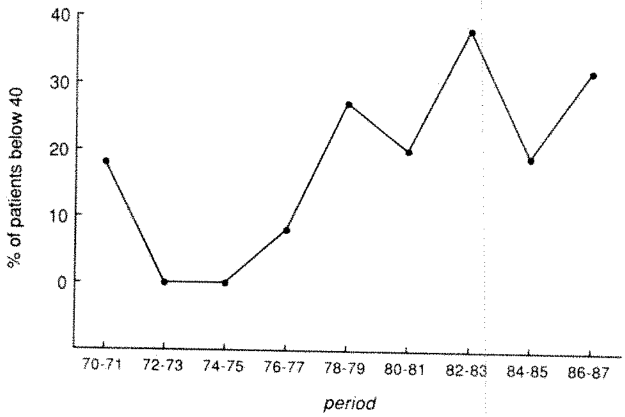

FIGURE 4. Percentage of patients with stage I melanomas below the age of 40 and year of presentation.

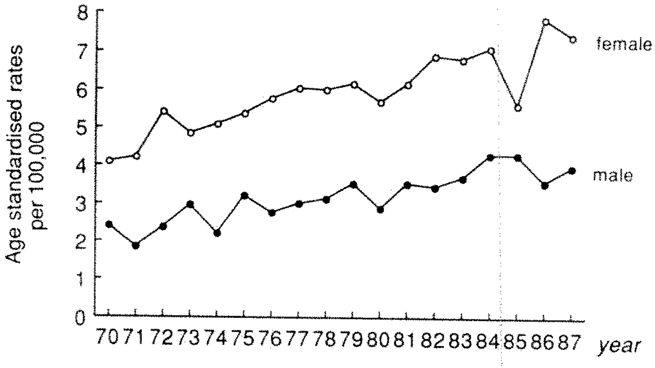

FIGURE 5. Age standardized rates for males and females with malignant melanoma in SE and SW Thames 1970–1987.

DISCUSSION

This study is unique in the extent of precampaign melanoma material that was examined. It suggests that the pathological characteristics of cutaneous melanoma have been changing over a long period, well before the introduction of our public education campaign for the early diagnosis of melanoma. Median melanoma thickness has been decreasing and the proportion of thin tumours (< 1·5 mm) has increased, accompanied by a concurrent fall in tumours of the thick category (> 3·5 mm). The greatest change in this trend was observed in the late 1970s.

The histological growth pattern of melanoma has also changed during the 18 years, with a relative increase in the proportion of tumours of the superficial spreading variant and less tumours of the nodular type. The proportion of patients under the age of 40 with melanoma has also increased.

These long-term trends have also been found by other studies world-wide. In a collaborative study of 1647 patients with melanoma from Alabama, U.S.A. and New South Wales, Australia, Balch *et al.*[10] have demonstrated a trend of decreasing median melanoma thickness since 1955, the largest decrease occurring long before the introduction of any large scale education campaign. A review of 4700 cases of cutaneous melanoma in The Netherlands during 1946–88[11] showed a similar gradual decrease in tumour thickness over the last 20 years, as did a study of 220 patients in Israel[12] and a study of over 2000 patients in Germany.[13] Balch *et al.*[10] also demonstrated a change in the distribution of the histological growth pattern with relatively more superficial spreading lesions and less nodular lesions in more recent years. This change also antedated their campaigns, as with our study.

The reason for these long-term trends is not clear. One possible explanation is that the biology of melanoma has been changing over the last 30 years. This would account for the observed changes in the histological characteristics of the tumours, as well as the universality of the change. This explanation seems more plausible in view of the changes in likely host/environmental aetiological factors, such as sun-exposure habits, increase in holidays in sunny climates, and a depletion of the ozone layer. Increased awareness and reporting of the disease is another factor in interpreting the observed trends, but it is unlikely that greater public[14] and professional[15] awareness alone can account for the changes in the histological growth patterns of melanoma or produce such long-term trends in so many different countries.

The large decrease in tumour thickness seen in the late 1970s in our study was also noted in the study from New South Wales and Alabama,[10] although other studies world-wide have shown a more gradual trend in decreasing tumour thickness over this period. Perhaps the increase of thin tumours in the late 1970s is therefore more likely to be due to a mass effect of public awareness rather than any major world-wide environmental event such as sun-spot activity.[16]

The aim of the study was to help us evaluate the effects of a public education campaign directed towards earlier diagnosis of melanoma. However, by demonstrating pre-existing trends in the parameters used to assess such campaigns, this has made our task of evaluation more difficult. Many more melanoma patients were seen during the campaign years, and more thin tumours were diagnosed in the months following the media campaigns of 1986 and 1987 (Fig. 2b). However, this cannot be attributed solely to the campaign, as such a seasonal variation, although less marked, was present in the 3 years prior to the campaign (Fig. 2b), and possibly reflects an increased tendency of individuals to examine their skin during the summer months when less clothing is worn. Although cases of melanoma-*in-situ* were not included in data, it is not known exactly what proportion of these lesions can progress to invasive melanoma. These 'melanoma precursors' are theoretically the ultimate in prognostic catch, and it is of interest that up until 1977, not a single melanoma-*in-situ* lesion was excised, yet during the campaign years, 10 such lesions (out of a total of 17 for the whole series) were removed, suggesting that the campaign may have a useful role in targeting this group of lesions.

Our public education campaign did not effect a noticeable increase in the reported melanoma incidence of the population targeted by the campaign. Figure 5 shows the age standardized rates (i.e. incidence per 100,000 population that would have occurred in a population of standard age and sex structure) for males and females in the south-east and south-west Thames area for 1970–1987. Although an overall increase in incidence was seen over the 18-year period, very little change in this trend occurred during the campaign years apart from an increase in the number of female melanomas in 1986.

Other centres in the U.K. have also experienced difficulties in evaluating the effectiveness of their campaigns, particularly because of a lack of suitable control groups. Such a problem existed with the Glasgow public education campaign,[4] where they elected to use 6 years of precampaign data (1978–1984) as an internal control. Their baseline data was unusual in not showing the trends of decreasing melanoma thickness seen throughout the world, but perhaps this was due to the geographical location, genetic susceptibility and holiday-exposure habits of the population sample studied.[17]

Retrospective studies such as ours are often criticized because of sampling error; i.e. older histological sections might not represent maximum tumour thickness as they were cut at a time when this was not routinely measured. At King's College Hospital there is a practice of taking central blocks from each specimen, and Søndergaard[18] has shown that this is likely to produce a fully representative section in over 95% of cases. Many old blocks were resectioned and even if some bias still existed, this can only underestimate the trends that we showed in tumour thickness as it is only possible to cut sections that are thinner than maximum tumour thickness, assuming correct orientation.

Demonstrating increased numbers of patients within assessment centres and increasing the proportion of thin tumours appears encouraging, but as yet no strong evidence supports the assumption that this will cause a reduction in the population mortality of melanoma.[19] It is possible that the campaigns are simply exhausting the population of easily recognizable lesions whose biological behaviour is more benign, increasing the bias of hospital based data.

The impact of the campaign upon the departmental workload was considerable, as other centres have found.[6] The anxiety caused by the campaign resulted in a considerable increase in the number of benign pigmented lesions (campaignomas) seen in routine and biopsy clinics causing longer waiting list times, although the melanoma pick-up rate of 1 per 18 benign pigmented lesions seen in the pigmented lesion clinic was encouraging.

Until mortality data show a clear benefit, the introduction of public education campaigns for the early diagnosis of melanoma remains controversial. The relative contribution of changing biology of melanoma versus the benefits of education campaigns will be difficult to disentangle. Any benefit in such a devastating disease is welcome.

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Growth-inhibitory effects of 1,25-dihydroxyvitamin D_3 on normal and psoriatic keratinocytes

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SUMMARY

The effects of 1,25-dihydroxyvitamin D_3 ($1,25(OH)_2D_3$) on the growth and DNA synthesis of cultured human keratinocytes obtained from involved and uninvolved psoriatic epidermis and normal epidermis were studied. Treatment with 10^{-8} M and 10^{-7} M of $1,25(OH)_2D_3$ inhibited cell growth as follows: 58·5 ± 19·3% and 21·3 ± 13·6% in normal keratinocytes ($n = 6$); 43·8 ± 22·8% and 17·8 ± 12·3% in psoriatic uninvolved keratinocytes ($n = 4$); 51·7 ± 18·2% and 13·2 ± 6·4% in psoriatic involved keratinocytes ($n = 6$). Inhibition was virtually complete at 10^{-6} M. DNA synthesis was also inhibited by 10^{-8} M, 10^{-7} M and 10^{-6} M of $1,25(OH)_2D_3$ as follows: 70·0 ± 8·3%, 59·0 ± 6·8% and 16·7 ± 4·0%, respectively, in normal keratinocytes ($n = 3$); 78·5 ± 13·5%, 51·5 ± 25·5% and 24·5 ± 21·5%, respectively, in psoriatic uninvolved keratinocytes ($n = 2$); and 69·3 ± 14·5%, 41·3 ± 19·1% and 14·8 ± 11·2%, respectively, in psoriatic involved keratinocytes ($n = 4$). These results indicate that $1,25(OH)_2D_3$ functions as a growth inhibitor for cultured human keratinocytes derived from both normal and psoriatic skin.

A biologically active form of vitamin D_3, 1,25-dihydroxyvitamin D_3 ($1,25(OH)_2D_3$), has been shown to modulate various cellular functions in different cells, including the epidermal keratinocyte. It has also been reported that $1,25(OH)_2D_3$ promotes the terminal differentiation of cultured murine epidermal cells[1] and that it inhibits DNA synthesis in human keratinocytes and stimulates their differentiation.[2] The presence of specific receptors for $1,25(OH)_2D_3$ was shown in human epidermis[3] and on cultured epidermal cells.[4] Recently we have characterized the growth inhibitory effects of $1,25(OH)_2D_3$ on normal human keratinocytes.[5] The growth inhibition was accompanied by changes related to proliferation, with a marked inhibition of DNA synthesis, a decrease in the number of high-affinity receptors for epidermal growth factor, and a rapid decrease in c-myc mRNA. It was confirmed by measuring the viability and protein synthesis of cells treated with $1,25(OH)_2D_3$ that this growth inhibition was not caused by a cytotoxic effect.

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Psoriasis is a common skin disorder that is characterized by a hyperproliferation of keratinocytes in the epidermis. Morimoto *et al.* first reported the clinical effectiveness of $1,25(OH)_2D_3$ on patients with psoriasis[6] and since then, several studies [7–10] have shown that oral administration or topical application of $1,25(OH)_2D_3$ and its analogues might be safe and effective therapy for this disease. The mode of action of $1,25(OH)_2D_3$ in the treatment of psoriasis is as yet not known. Fibroblasts from psoriatic skin show a partial resistance to the antiproliferative effect of $1,25(OH)_2D_3$.[11] In this study the growth inhibitory effect of $1,25(OH)_2D_3$ was studied on keratinocytes from normal and from the uninvolved and involved skin of patients with psoriasis.

METHODS

Cell culture

Samples of normal skin were obtained from that excised during plastic surgery, and biopsies were taken from involved and uninvolved areas of psoriasis. Human keratinocytes were cultured using a modification of the technique of Wille *et al.*[12] Briefly, skin was cut into 3–5 mm pieces, and floated in dispase solution (500 U/ml) overnight at 4°C.[13] After separation of the epidermis from the dermis, the epidermal sheets were rinsed with Ca^{2+}- and Mg^{2+}-free phosphate-buffered saline (PBS) and incubated in a 0·25% trypsin solution for 10 min at 37°C and the epidermis then teased apart with forceps. Epidermal cells were suspended in nutrient medium MCDB153 (Kyokuto Co., Tokyo, Japan) supplemented with EGF (10 ng/ml), insulin (5 μg/ml), hydrocortisone (5×10^{-7} M), ethanolamine (0·1 mM), phosphoethanolamine (0·1 mM) and bovine hypothalamic extract (150 μg/ml).[14] The stock solution consisted of MCDB153 supplemented with 0·1 mM Ca^{2+} plus elevated concentrations of amino acids (0·75 mM isoleucine, 0·24 mM histidine, 0·09 mM methionine, 0·09 mM phenylalanine, 0·045 mM tryptophan, 0·075 mM tyrosine) and antibiotics (100 IU/ml penicillin and 100 μg/ml streptomycin). The cells were plated at a density of 10^5/ml in 28-cm^2 plastic plates coated with collagen (Iwaki Glass, Tokyo, Japan). EGF was omitted for several days after plating. After reaching subconfluency, the cells were subcultured. Cells were washed once with PBS and then incubated with EDTA/trypsin solution for 5 min at 37°C. An equal volume of MCDB153 medium containing 20% (v/v) FCS was added and the mixture was centrifuged at 200 ***g*** for 5 min. Cell pellets were washed with MCDB153 medium and plated. Second-passage cells were used in this experiment. Unless otherwise indicated, keratinocytes were plated in six-well plates at a density of $1·5 \times 10^4$ cells/well. $1,25(OH)_2D_3$ was solubilized in ethanol, and the same concentration of ethanol was used in controls.

Measurement of cell growth

Keratinocytes were plated in six-well plates at a density of 10^4 cells/well, and the next day the medium was changed and an appropriate amount of $1,25(OH)_2D_3$ was added. After the growth period, usually 1 week, the cells were fixed in 10% formalin in PBS and stained with 0·2% crystal violet in 0·1 M citric acid. The total area stained was measured by a computer-aided video-camera and image processor system.[15] Briefly, stained cells were photographed with a 3-tube video-camera (Ikegami Tsushinki Co., Tokyo, Japan, Model ITC-730). The image was processed by Nexus 6400 (Kashiwagi Res. Corp., Tokyo) and the transformed information was sent to a computer (NEC, Tokyo, Model PC-9801-VM2) which calculated the percentage of stained area compared to total area. The cell growth was usually expressed as the percentage of stained area relative to the stained area of the controls. In several experiments, cell growth was calculated by this method as well as the conventional method of cell counting using a haemocytometer, and results from both methods were the same.

Measurement of DNA synthesis

DNA synthesis of keratinocytes was measured by the incorporation of $[^3H]$-thymidine. Keratinocytes were plated in 24-well trays at 5×10^4 cells/well. After reaching subconfluency the cells were treated with various concentrations of $1,25(OH)_2D_3$ for 48 h, and pulse-labelled with 1 μCi of methyl-$[^3H]$-thymidine (25 μCi/mmol, CEA) for 3 h. Cultures were then washed three times with PBS containing 1 mg/ml of bovine serum albumin and twice with 10% (w/v) trichloroacetic acid. Cells were solubilized with 1 M NaOH and the radioactivity counted in a scintillation counter. The averages of triplicate measurements were calculated.

RESULTS

Effect of $1,25(OH)_2D_3$ on the growth of keratinocytes

Psoriatic keratinocytes from involved lesions were cultured in the presence or absence of various concentrations of $1,25(OH)_2D_3$ for 1 week and stained with crystal violet (Fig. 1). The growth of these keratinocytes was inhibited by $1,25(OH)_2D_3$ at concentrations higher than 10^{-8} M. To determine whether the responsiveness of normal and psoriatic keratinocytes to growth inhibition by $1,25(OH)_2D_3$ differs, the effect of $1,25(OH)_2D_3$ on keratinocyte growth was studied using cultured keratinocytes obtained from skin samples of six normal individuals, as well as from four uninvolved and six involved areas of patients with psoriasis.

The growth of normal keratinocytes incubated with 10^{-8} M and 10^{-7} M of $1,25(OH)_2D_3$ was

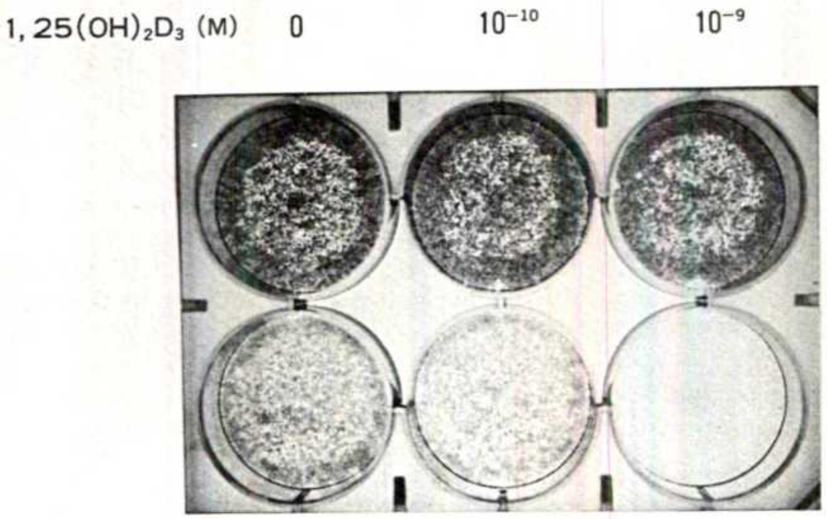

FIGURE 1. Effect of $1,25(OH)_2D_3$ on the growth of psoriatic involved keratinocytes cultured in a serum-free condition. Keratinocytes were cultured in the presence of various concentrations of $1,25(OH)_2D_3$ for 1 week then stained with crystal violet.

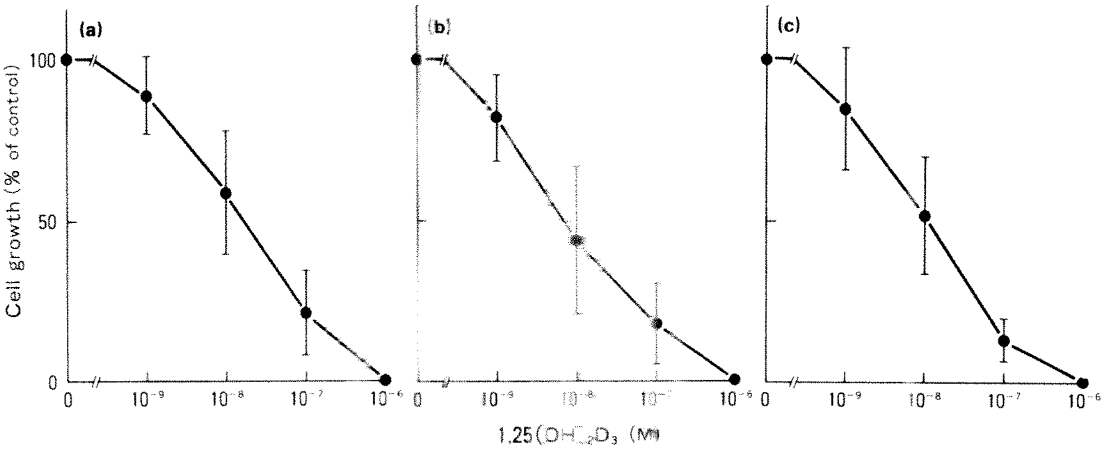

FIGURE 2. Effect of $1,25(OH)_2D_3$ on the growth of human keratinocytes from normal and psoriatic skin samples. The cell growth is expressed as a percentage of the control. Each point shows the mean (± SD). (a) Normal keratinocytes, $n = 5$; (b) psoriasis uninvolved keratinocytes, $n = 4$; (c) psoriasis involved keratinocytes, $n = 6$.

reduced to $58{\cdot}5 \pm 19{\cdot}3\%$ and $21{\cdot}3 \pm 13{\cdot}6\%$ of control values (Fig. 2a); the growth of keratinocytes from psoriatic uninvolved epidermis was reduced to $43{\cdot}8\% \pm 22{\cdot}8\%$ and $17{\cdot}8 \pm 12{\cdot}3\%$, respectively (Fig. 2b). The growth of keratinocytes from psoriatic involved epidermis was reduced to $51{\cdot}7 \pm 18{\cdot}2\%$ and $13{\cdot}2 \pm 6{\cdot}4\%$, respectively (Fig. 2c). The growth inhibition of the keratinocytes by $1,25(OH)_2D_3$ was virtually complete at a concentration of 10^{-6} M.

Effect of 1,25(OH)$_2$D$_3$ on DNA synthesis

The effect of $1,25(OH)_2D_3$ on DNA synthesis in keratinocytes was studied using the incorporation of [^{3}H]-thymidine in skin samples of three normal individuals, and from two areas of uninvolved skin and four areas of involved skin of four patients with psoriasis. The incorporation of the [^{3}H]-thymidine was inhibited in a dose-dependent manner by the addition of $1,25(OH)_2D_3$ (Fig. 3). The incorporation was decreased to $70{\cdot}0 \pm 8{\cdot}3\%$, $59{\cdot}0 \pm 6{\cdot}8\%$ and $16{\cdot}7 \pm 4{\cdot}0\%$ of control values at 10^{-8} M, 10^{-7} M and 10^{-6} M of $1,25(OH)_2D_3$, respectively, in normal keratinocytes (Fig. 3a). In keratinocytes from the uninvolved skin in psoriasis the incorporation was reduced to $78{\cdot}5 \pm 13{\cdot}5\%$, $51{\cdot}5 \pm 25{\cdot}5\%$ and $24{\cdot}5 \pm 21{\cdot}5\%$ by treatment with $1,25(OH)_2D_3$ at 10^{-8} M, 10^{-7} M and 10^{-6} M, respectively (Fig. 3b). Treatment of keratinocytes from the involved skin with $1,25(OH)_2D_3$ resulted in a reduction of [^{3}H]-thymidine incorporation to $69{\cdot}3 \pm 14{\cdot}5\%$, $41{\cdot}3 \pm 19{\cdot}1\%$ and $14{\cdot}8 \pm 11{\cdot}2\%$ at 10^{-8} M, 10^{-7} M and 10^{-6} M, respectively (Fig. 3c).

DISCUSSION

In the present study, a comparison of the growth-inhibitory effects of $1,25(OH)_2D_3$ was carried out on cultured keratinocytes from normal skin and on the uninvolved and involved skin of patients with psoriasis. Smith *et al.* had difficulty in culturing keratinocytes from the involved skin of psoriasis,[10] but in our study there was no difficulty.

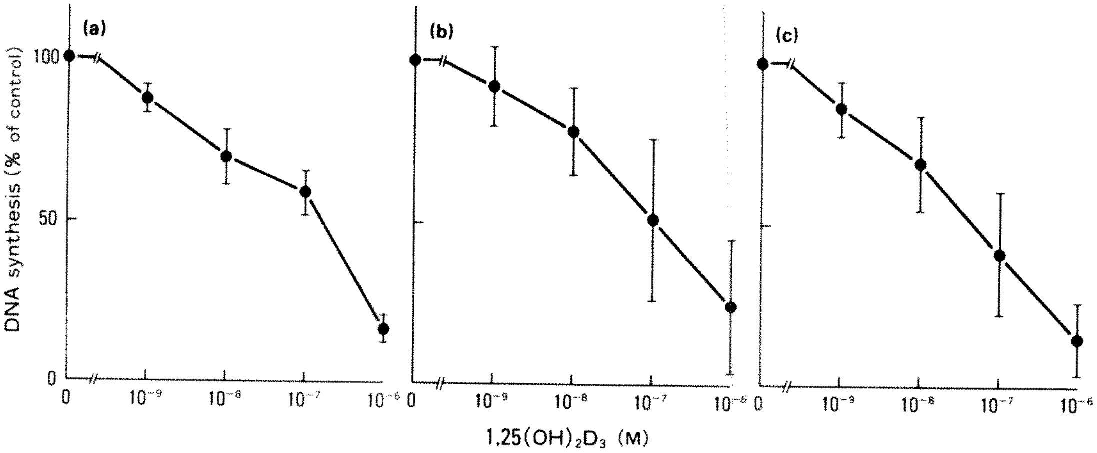

FIGURE 3. Effect of $1,25(OH)_2D_3$ on the DNA synthesis of human keratinocytes from normal and psoriatic skin samples. Cells cultured with vehicle (0·1% ethanol) alone were used as a control. Values are expressed as a percentage of the control. Each point shows the mean (± SD). (a) Normal keratinocytes ($n = 3$); (b) psoriasis uninvolved keratinocytes ($n = 2$); (c) psoriasis involved keratinocytes ($n = 4$).

Psoriasis is a hyperproliferative disorder of keratinocytes, but abnormal behaviour of the fibroblasts has been reported.[10,11,16] It is possible that the fibroblasts respond to $1,25(OH)_2D_3$ in an abnormal manner and modulate the growth of psoriatic keratinocytes. Fibroblasts from the involved and uninvolved skin of patients with psoriasis were found to be resistant to growth inhibition by $1,25(OH)_2D_3$.[11] More recently, however, the same group found resistance to $1,25(OH)_2D_3$ in only two fibroblast cultures out of five patients that were studied.[10] Kreuger *et al.* reported that none of eight involved and uninvolved psoriatic fibroblast lines differed from four normal fibroblast lines, and that all the cultures of fibroblasts were inhibited by $1,25(OH)_2D_3$ in a dose-dependent manner.[17] These results indicate that resistance to the antiproliferative effect of $1,25(OH)_2D_3$ is not common to all psoriatic fibroblasts.

In this study we investigated the inhibitory effect of $1,25(OH)_2D_3$ on the cell growth and DNA synthesis using cultured keratinocytes from involved and uninvolved skin of patients with psoriasis. The results indicated that cultured keratinocytes from both involved and uninvolved skin were not resistant to the antiproliferative effect of $1,25(OH)_2D_3$. DNA synthesis was inhibited by $1,25(OH)_2D_3$ in the same manner as keratinocytes from normal epidermis. Resistance to the antiproliferative effect and DNA synthesis inhibition of $1,25(OH)_2D_3$ was not found in any of the psoriatic keratinocytes studied. Smith *et al.* also reported no resistance in keratinocyte cultures from psoriatic skin,[10] although they examined only two cultures. In contrast keratinocytes of explant cultures of keratinocytes from psoriatic skin were reported to be resistant to inhibition of DNA synthesis by $1,25(OH)_2D_3$, and that a more than 100-fold amount of $1,25(OH)_2D_3$ was required to inhibit DNA synthesis in keratinocytes from both involved and uninvolved skin.[18] The discrepancy between their results and ours may be due to differences in the culture methods. They used explant out-growth culture which needs a feeder layer of fibroblasts and these cells may influence keratinocytes in a paracrine fashion. Fetal calf serum present in their explant out-growth culture may modulate the effect of $1,25(OH)_2D_3$. However, the most distinct difference is that explant out-growth culture is a primary culture, whereas the cells used in our method were passaged and the changes in psoriatic keratinocytes may be reversed. These findings indicate that resistance of psoriatic keratinocytes to antiproliferation by 1,25(OH)$_2$D$_3$ is not an inherent characteristic, but only a temporary phenomenon generated under psoriatic conditions.

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Psoriasis consultation audit: a two-centre study

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SUMMARY

The adequacy of hospital note-keeping in 100 patients presenting with psoriasis at two teaching hospitals has been assessed. The criteria used were defined in consultation with a peer group of 60 British dermatologists. The pattern of record keeping was similar in both centres. Where differences existed between consultant (C) and non-consultant (J) staff, non-consultant staff completed the records more fully. There were differences between centres in the recording of patients' symptoms and disability, psoriasis type, and in the number of visits before discharge to general practitioner care. Both centres had a poor record of communication to general practitioners following patient default from the clinic. In both centres, the patient had only a one in 8 chance of subsequently being seen by a consultant if at the first visit they had been seen by a non-consultant. This simple case-note audit has highlighted the similarity of practice in the two centres and has resulted in practical improvements in record keeping.

Although measures of the outcome of different treatment strategies rely largely on the completeness of hospital note-keeping, little attention has been directed to this aspect of management. In the hospital, where there are often frequent changes of junior staff, effective patient care relies upon the accuracy of medical records. The standard of note-keeping is important for both clinical and administrative (including medico-legal) purposes, but the criteria by which this can be judged are rarely established.

We have defined a criterion set from which the adequacy of note-keeping could be assessed in patients suffering from psoriasis. The criterion set was established in conjunction with a peer group of practising dermatologists and used to compare the performance of two teaching hospital departments of dermatology.

METHODS

The criterion set used in this analysis was derived at three workshop meetings during 1988,

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involving 60 British dermatologists from throughout the U.K. Participants were asked to define up to 20 items of information (criteria) which they considered should be the minimum recorded in the case notes of a patient presenting with psoriasis. The information to be included was not necessarily confined to medical details; information of a more generally administrative or operational nature was allowed if there was agreement that it was a medical responsibility to ensure that such data was recorded.

After each of the workshop meetings, pilot studies were conducted and minor modifications made as to the content and style of the criterion set. The final document used in the study consisted of 17 criteria, covering the following areas: first visit, history, examination, diagnosis, management, and follow-up (Table 1). The precise interpretation of specific criteria, agreed before the study is shown in the key to Table 1.

Ethical permission for the study was obtained in each of the two centres. The case notes of 100 patients presenting with psoriasis were drawn at random from the diagnostic indices of new out-patients at the University Hospital of Wales, Cardiff, and the Royal Infirmary, Glasgow. During 1988, doctors in both centres were considering the question of psoriasis audit, and therefore only the notes of patients presenting in 1987 were considered. Each set of notes was audited by a consultant and senior registrar. Both the handwritten record and the letter back to the referring doctor were consulted as sources of information.

The grade of doctor encountered at the first patient visit was also recorded, either as consultant (C) or non-consultant (J). Junior staff were of registrar or senior registrar grade in both centres. In addition, for the purposes of this study, other staff regarded as 'non-consultant' included in one centre a senior house officer and clinical assistant, and in the other centre an associate specialist. Each criterion was recorded as being 'present', 'absent' or 'not applicable'. Where the data allowed, a comparison was made between the combined scores of the consultant and non-consultant staff from both centres.

The results were analysed using the χ^2 test.

RESULTS

The participating centres have been designated A and B to preserve anonymity.

Overall results combined for both centres

The combined results are shown in Table 2 (column 3). The pattern of record keeping was broadly similar in both centres. Performance was generally satisfactory in recording the date of first attendance, reference to current and/or previous drug therapy, and the distribution of the patient's rash. However, there was a failure to record basic data and there was evidence of deficiencies in clinical practice.

Failure to record data

In the majority of case notes, there was no reference to the patient's symptoms or to the disability caused by their condition. Although the performance of both centres was poor, centre B performed significantly better than centre A ($P < 0·001$). Both centres rarely recorded any subsequent change in symptoms or disability at follow-up. (Criterion 15 was considered to be 'not applicable' if the patient was discharged after the first visit, or if no assessment had been recorded for criterion 4.)

Very few records from either centre contained information on the extent of the psoriasis. One of the centres routinely included a body map in the case notes to facilitate the recording of this

TABLE 1. Criterion set used in the study

Designation of record keeper.................................
Hospital number...........
Date first seen...........

Analysis of the hospital record identifies	Present	Absent	N/A
First visit			
1 Clinic visit date	....	....	....
2 Entry signed by doctor	....	....	....
3 Consultant in charge of case identified	....	....	....
History			
4 Patient's symptoms or psoriasis disability	....	....	....
5 Reference to current and/or previous drug therapy	....	....	....
Examination			
6 Distribution of rash	....	....	....
7 Extent of disease*	....	....	....
Diagnosis			
8 Particular type of psoriasis†	....	....	....
Management			
9 Drug or physical treatment specified	....	....	....
10 Record of written information given to patient	....	....	....
11 Letter to referring physician within 7 days of clinic	....	....	....
First follow-up			
12 (a) Any assessment of progress noted	....	....	....
12 (b) Patient's assessment of progress noted	....	....	....
13 If on second line therapy,‡ haematology and biochemistry results correctly filed	....	....	....
14 If patient did not attend, GP informed	....	....	....
15 Statement of change in perceived disability or symptoms	....	....	....
Subsequent records			
16 Record of assessment by a consultant	....	....	....
17 Patient discharged to GP within five clinic visits	....	....	....

N/A, not applicable.
* Either graphically or by estimate of percentage involvement.
† 'Type of psoriasis' should specify one of: plaque, guttate, erythrodermic, pustular, nail, joint, scalp.
‡ 'Second line therapy' includes: methotrexate, etretinate, cyclosporine, azathioprine, hydroxyurea.

data, but it was rarely used. The type of psoriasis was more frequently recorded in centre B than centre A ($P < 0{\cdot}001$). Overall, this information was absent from 42·5% of case notes.

Some assessment of progress was recorded in the vast majority of case notes, but it was often difficult to determine whether the assessment was that of the doctor or the patient. Many patients were not routinely followed-up after the initial consultation, and therefore information on their progress was unavailable.

TABLE 2. Comparison of criterion set scores between the two centres

Criterion no.	(1) Centre A (%) P	(1) Centre A (%) A	(1) Centre A (%) N/A	(2) Centre B (%) P	(2) Centre B (%) A	(2) Centre B (%) N/A	(3) Total (%) P	(3) Total (%) A	(3) Total (%) N/A	(4) Difference between centres P (χ^2 test)
1	97	3	0	100	0	0	98·5	1·5	0	NS
2	33	67	0	25	75	0	29	71	0	NS
3	71	29	0	75	25	0	73	27	0	NS
4	17	83	0	50	50	0	33·5	66·5	0	$< 0{\cdot}001$ ($\chi^2 = 24{\cdot}4$)
5	77	23	0	78	22	0	77·5	22·5	0	NS
6	83	17	0	91	9	0	87	13	0	NS
7	3	97	0	5	95	0	4	96	0	NS
8	46	54	0	69	31	0	57·5	42·5	0	$< 0{\cdot}001$ ($\chi^2 = 11{\cdot}4$)
9	99	1	0	98	2	0	98·5	1·5	0	NS
10	4	96	0	9	90	1	6·5	93	0·5	NS
11	88	11	1	98	2	0	93	6·5	0·5	NS
12a	46	4	50	62	7	31	54	5·5	40·5	NS
12b	5	45	50	5	64	31	5	54·5	40·5	NS
13	2	2	96	0	0	100	1	1	98	NS
14	0	18	82	0	21	79	0	19·5	80·5	NS
15	1	3	96	8	26	66	4·5	14·5	81	NS
16	61	39	0	54	45	1	57·5	42	0·5	NS
17	65	16	19	37	12	51	51	14	35	$< 0{\cdot}001$ ($\chi^2 = 22{\cdot}9$)

P, present (i.e. data recorded). NS, not significant.
A, absent (i.e. data not recorded).
N/A, not applicable.

Deficiencies of practice.

The majority of clinical records in both centres were unsigned at the initial visit, and in 27% of the case notes, the consultant in charge of the case was not identified. In practice, this usually meant that no consultant had been designated by the administrative staff.

In both centres, the drugs or physical treatment prescribed at the initial consultation were nearly always specified in the case notes. However, mention was rarely made in either centre of what printed or written information had been given to the patient regarding their treatment. Both centres had a good record of communicating with the general practitioner after the first consultation, and most letters were sent out within 7 days of the clinic visit. However, if the patient did not attend for their follow-up appointment, neither centre routinely relayed this information to the general practitioner.

Evidence of any consultant involvement was lacking in 42% of records and the results were similar for both centres. The patient had only a one in 8 chance of subsequently being seen by a consultant if, at first visit, they had seen a non-consultant (Table 3, column 2).

Overall, 51% of patients were discharged back to their general practitioner within five clinic visits. A further 35% of patients failed to attend for follow-up within that period. Centre A performed significantly better than centre B in discharging patients within five clinical visits ($P < 0{\cdot}001$) and fewer patients defaulted from follow-up.

Comparison between consultant and non-consultant staff

The results are shown in Table 3. In centre A, 56 records had been completed by consultant

TABLE 3. Comparison of criterion set scores between consultants and non-consultants

Criterion no.	(1) Consultants %			(2) Non-consultants %			(3) Difference between grades
	P	A	N/A	P	A	N/A	P (χ^2 test)
1	98	2	0	99	1	0	NS
2	18	82	0	41	59	0	$<0{\cdot}001\ (\chi^2=13{\cdot}4)$
3	96·5	3·5	0	69	31	0	N/A
4	25	75	0	41	59	0	$<0{\cdot}02\ (\chi^2=5{\cdot}6)$
5	71	29	0	85	15	0	$<0{\cdot}02\ (\chi^2=6{\cdot}4)$
6	78	22	0	95	5	0	$<0{\cdot}001\ (\chi^2=13{\cdot}3)$
7	1	99	0	7	93	0	$<0{\cdot}05\ (\chi^2=4{\cdot}9)$
8	39	61	0	77	23	0	$<0{\cdot}001\ (\chi^2=29{\cdot}6)$
9	97	3	0	100	0	0	NS
10	5	95	0	8	92	0	NS
11	95	4	1	89	11	0	NS
12a	—	N/A	—	—	N/A	—	N/A
12b	—	N/A	—	—	N/A	—	N/A
13	—	N/A	—	—	N/A	—	N/A
14	—	N/A	—	—	N/A	—	N/A
15	—	N/A	—	—	N/A	—	N/A
16	100	0	0	13	87	0	N/A
17	48	24	28	54	3	43	$<0{\cdot}001\ (\chi^2=15{\cdot}1)$

P, present (i.e. data recorded).
A, absent (i.e. data not recorded).
N/A, not applicable.

staff, and 44 by non-consultant staff. For centre B, the figures were 47 and 53, respectively. Because patients may have been seen by a different grade of staff at subsequent visits, criteria 12–15 (which relate to follow-up), have not been separately analysed. In general, non-consultant staff completed the records more fully than their consultant colleagues. This was true in respect of signature of record entry, record of symptoms or disability, record of previous drug therapy, distribution and extent of rash, and type of psoriasis. In addition, discharge back to the general practitioner within five clinic visits was more likely if the patient was initially seen by a non-consultant.

DISCUSSION

This study revealed a number of deficiencies in the recording of data, communication with general practitioners, and some aspects of patient management. The pattern of record keeping in the two centres was very similar. This could not be explained on the basis of previous shared experience or practice as none of the staff from centre A had ever worked with staff from centre B.

The absence of any reference to patient's symptoms or disability in most of the case notes studied may seem surprising. The prime concern of many patients with psoriasis is the cosmetic disability which even in mild cases may be sufficient to prevent normal social activity. This is rarely documented by dermatologists. In addition, there is difficulty in assessing the disability, either physical or psychological, in a way that is easily quantifiable and reproducible. The simple assessments of function available to other specialities are difficult to construct in dermatology. Attempts have been made to develop a disability index for psoriasis[1] and acne,[2] but these are not widely used, and are time consuming in a busy out-patient clinic. A simple standard abbreviated disability questionnaire is currently being evaluated by us.

In the vast majority of case notes studied, it was impossible to determine the extent or distribution of psoriasis. The availability of body maps in the case notes of one centre did not improve the recording of this data. Many clinicians must rely on the patient's assessment of their response to treatment, rather than on more objective measures. Once again, however, we found it difficult to determine whose assessment of treatment (i.e. patient or doctor), was recorded in follow-up notes. Statements of change in perceived disability were almost always absent from follow-up notes, usually because no baseline had been established at the initial visit. The failure to record such essential data has serious implications for any future audit of outcome relating to different treatment strategies in this group of patients.

The study also revealed a number of areas in which there were defects in either administrative or medical practice. Case notes were frequently unsigned, especially if the patient had initially seen a consultant. In addition, 25% of case notes had no record of the consultant in overall charge. This was most likely to occur if a patient was simply referred to the department rather than to a specific named consultant. Both these deficiencies could have potentially serious medico-legal implications.

There appeared to be no consultant involvement in the management of 42% of patients studied, and only 13% of patients who saw a member of the junior staff at the first visit was subsequently seen by a consultant. These findings are similar to those reported in other specialities.[3] However, using the criterion set as a measure of performance, the junior staff were either equal to or better than their senior colleagues both in the recording of data and in aspects of management such as discharge back to the general practitioner. This may in part be due to an element of 'self audit' amongst the junior staff, who are aware that their note-keeping may be reviewed by a consultant, and therefore record data in more detail to avoid possible criticism.

With conventional topical therapy for psoriasis, some form of pre-printed written information is often helpful for a patient, by giving advice on the correct method of application of the treatment and warning about possible side-effects. Very few records in either centre indicated that such written information had been given. Usually, most of the information given to patients is in verbal form, but recording what information has been given may be of value if the patient is subsequently seen by another doctor.

Communication with general practitioners was generally satisfactory if the patient had attended the clinic, but neither centre had a policy of writing to the general practitioner when a patient failed to attend for follow-up. This deficiency is easily remedied once identified, but without some type of formal review would probably have remained undetected. The use of a criterion set as a means of auditing medical performance has not previously been reported in the U.K., but has been described in Australia.[4] Other British studies have drawn attention to the inadequacy of hospital case records,[5,6] and a recent report has shown how the use of guidelines can improve efficiency in a medical out-patient clinic.[7]

Whilst there may be no clear correlation between clinical performance and record keeping, our criterion set was designed to test only whether the minimum acceptable 'core data' was present in any individual set of case notes. It is difficult to see how patient management could be successfully assessed when so much of this 'core data' was missing.

The criterion set described could also be used, with minimal alteration, in assessing the adequacy of record keeping in other skin diseases such as eczema or acne. The information assessed is so basic that only simple changes would be required to allow its wider use in other specialities. We intend to repeat the audit in the near future to establish what improvements, if any, have occurred as a result of this exercise.

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We are grateful to Mr Howard May of Roche Products Ltd for providing an educational grant to fund the workshop meetings, and to Dr Robin Brooks of Vismed for organizing them. We wish to thank the dermatologists who helped with the construction of the criterion set, and our colleagues who allowed us to inspect their case notes and report the results.

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Subcutaneous xanthomatosis

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SUMMARY

A patient with multiple subcutaneous xanthomata in whom there were only minor abnormalities of the fasting serum lipid profile is described. To our knowledge, this is only the second published case of subcutaneous xanthomatosis.

Cutaneous and tendon xanthomata are well recognized manifestations of hyperlipoproteinaemia, of which there are several familial types.[1] In addition, a number of examples of normolipaemic xanthomatosis have been described, including cerebrotendinous xanthomatosis, xanthomata associated with underlying lymphoproliferative disorders and xanthoma disseminatum.[2] There has been one report of a case of normolipaemic subcutaneous xanthomatosis in a patient with diabetes mellitus and vocal cord xanthomata.[3]

We describe a patient with subcutaneous xanthomatosis, in the absence of diabetes mellitus and vocal cord xanthomata, in whom there were only minor abnormalities of the fasting serum lipid profile.

CASE REPORT

A 67-year-old man first presented in April 1987, having developed numerous asymptomatic, deep-set lumps on the back and proximal limbs during the preceding few months. During the next year, he developed large subcutaneous nodules on the abdomen and smaller lesions with overlying erythema on the chest. He was otherwise fit and well with an unremarkable past medical history, apart from a peptic ulcer over 30 years previously. There was no family history of any diseases, in particular diabetes mellitus.

Examination revealed numerous firm subcutaneous nodules over the right scapular region and on the upper arms, thighs and abdomen, some of which were a few centimetres in size (Fig. 1). There was reticulate erythema with underlying palpable infiltration on the chest (Fig. 2) and similar lesions on the upper back. He also had toe-nail dystrophy suggestive of fungus infection. Neurological examination revealed generalized diminished pin-prick sensation, but with no evidence of traumatic injury to the skin or joints.

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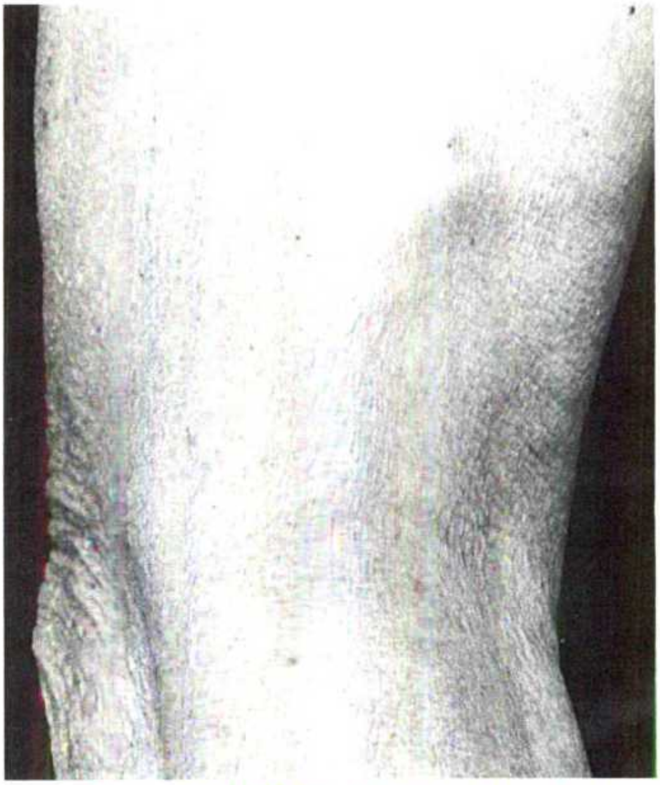

FIGURE 1. A large subcutaneous nodule on the right upper arm.

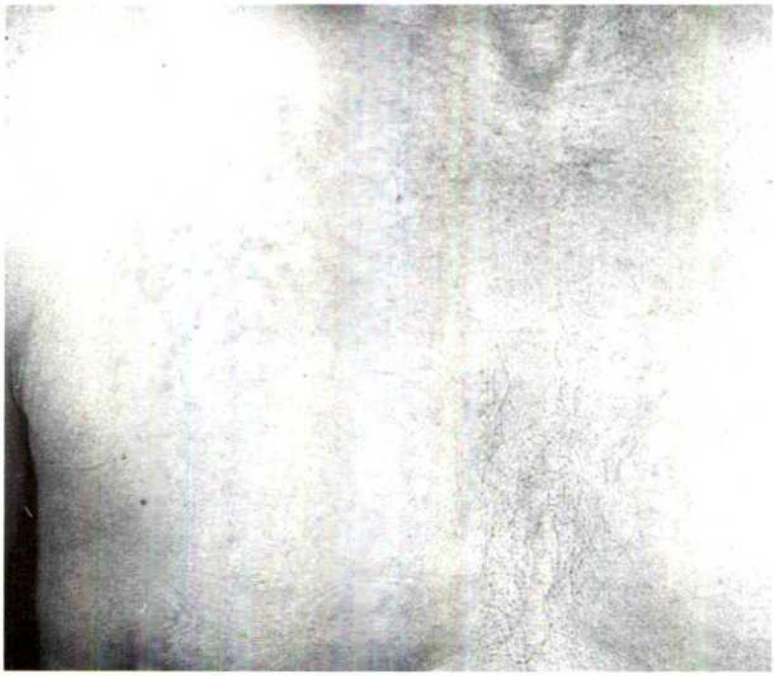

FIGURE 2. Reticulate erythema with underlying palpable infiltration on the chest.

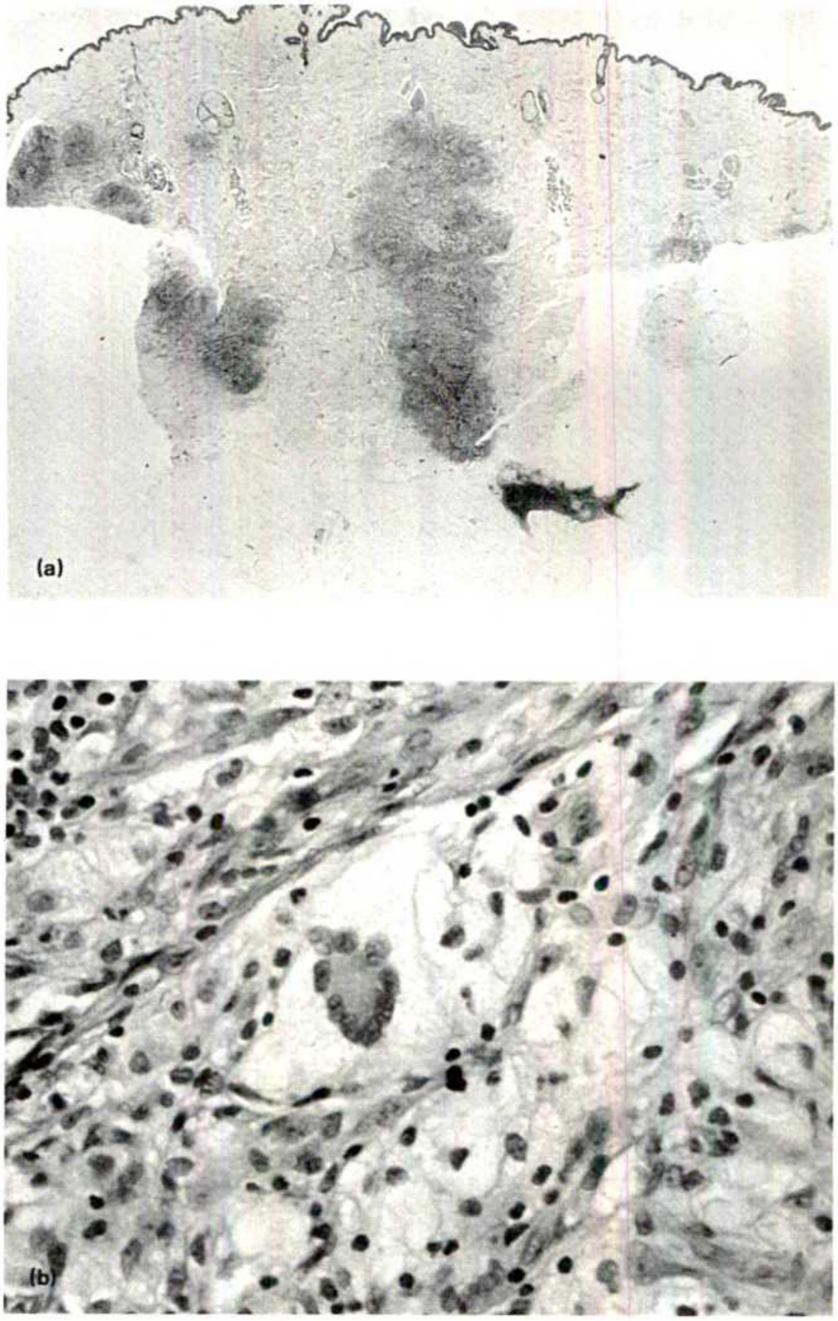

FIGURE 3. Haematoxylin and eosin-stained sections from a xanthoma from the right scapular region showing (a) a focal dermal infiltrate extending to the deep margin; and (b) a Touton giant cell with an infiltrate of foam cells and inflammatory cells.

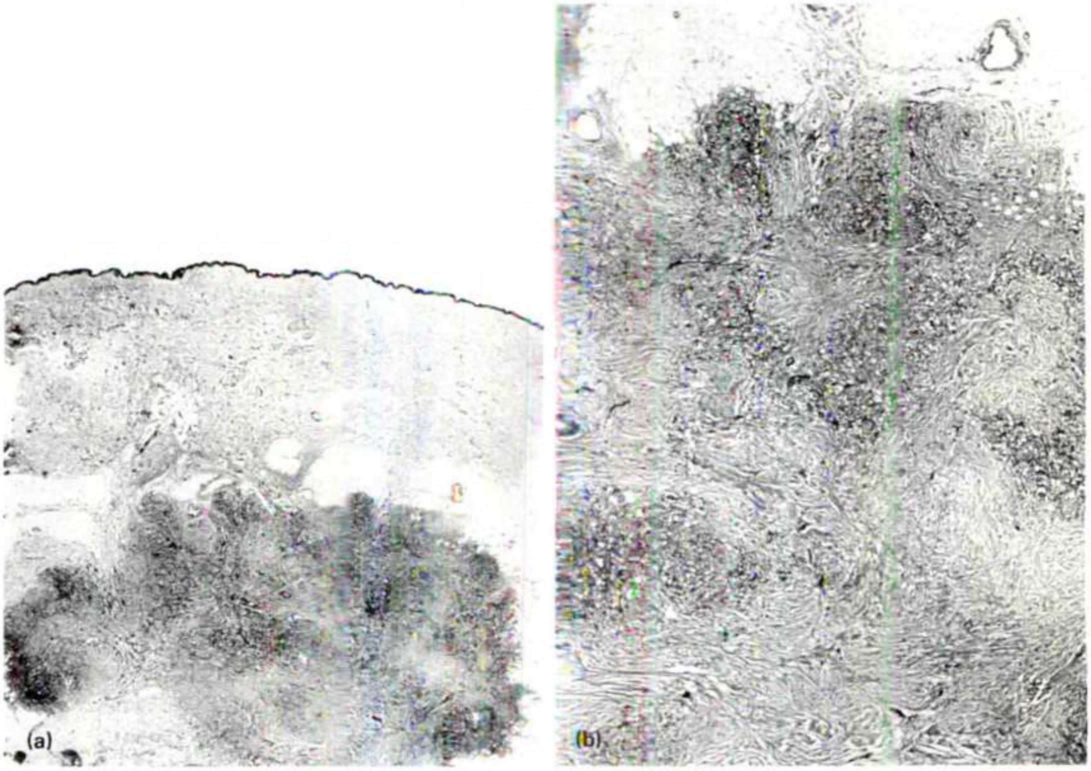

FIGURE 4. Haematoxylin and eosin-stained sections from the right arm nodule showing (a) an infiltrate almost exclusively below the subcutis comprising (b) areas of fibrosis surrounding islands of xanthomatous and inflammatory cells.

Investigations

A number of skin biopsies were performed. Histopathological examination of a nodule from the right scapular region showed a focal dermal infiltrate extending to the deep margin of the biopsy (Fig. 3a). The infiltrate comprised foam cells and Touton giant cells in addition to inflammatory cells (Fig. 3b). A biopsy from the arm lesion (see Fig. 1) revealed a similar infiltrate almost exclusively below the subcutis with areas of fibrosis surrounding islands of xanthomatous and inflammatory cells (Fig. 4a and b). The fat stain, Oil Red O, positively labelled the contents of the foamy macrophages in sections of frozen tissue. A biopsy of an area of reticulate erythema on the chest showed similar pathology in the mid-dermis.

A full blood count, erythrocyte sedimentation rate, routine biochemical screen, fasting and random blood glucose levels and immunoglobulins and electrophoresis were all normal. In view of the possibility of a lymphoproliferative disorder, bone marrow (both aspirate and trephine) biopsies and an abdominal CT scan were performed and these were all normal. Indirect laryngoscopy showed no evidence of vocal cord or hypopharyngeal xanthomata. The plasma alkaline phosphatase level was increased at 26 KA/dl (normal < 11 KA/dl). Isoenzymes confirmed this to be of bone origin and a chest X-ray showed the changes of Paget's disease of the scapula.

The fasting serum lipid profile was initially normal with a triglyceride level of 1·6 mmol/l and

TABLE 1. Quantitative analysis of the serum lipoprotein fraction

	Triglycerides (mmol/l)	(Normal)	Cholesterol (mmol/l)	(Normal)
VLDL	1·8*	(< 1·6)	0·8	(< 1·2)
LDL	—	—	4·0	(< 5·0)
HDL	—	—	0·7*	(> 1·05)
Total	2·89*	(< 2·6)	5·6	(< 6·7)

* Abnormal values.
—, Not estimated.

cholesterol level of 5·4 mmol/l (normal values for laboratory: triglycerides < 2·0 mmol/l, cholesterol < 6·5 mmol/l). Subsequent investigation revealed minor abnormalities in the serum lipid profile: total, very low density lipoprotein (VLDL) and low density lipoprotein cholesterol levels were normal but total and VLDL triglyceride levels were slightly increased and the high density lipoprotein (HDL) cholesterol level was reduced (Table 1).

With regard to the generalized diminished pin-prick sensation, neurological investigations have all been normal and our patient was considered to have a congenitally high pain threshold, unrelated to the xanthomatosis. *Trichophyton rubrum* was cultured from the nail clippings, but the patient was not treated with antifungal drugs.

Clinical course

The patient's skin signs remained unchanged during follow-up in the clinic from March 1988 onwards and he continued to be well. However, in November 1988 he complained of anorexia, nausea, weight loss and lumbar pain. A diagnosis of carcinoma of the rectum was made and an anterior resection of the rectum was performed. Eleven days after his operation the patient complained of abdominal pain and was found to have developed renal failure with a plasma urea of 16·7 mmol/l and creatinine of 159 μmol/l. He died suddenly on the following day. At the post-mortem there was focal infarction of the small bowel with a paralytic ileus and pulmonary oedema. The coronary arteries showed atherosclerotic narrowing but there was no evidence of recent thrombotic occlusion.

DISCUSSION

The normocholesterolaemic xanthomatoses have been subclassified into three groups, designated types I, II and III.[2] In type IA there is an accumulation of unusual lipids in the lipoprotein, as occurs, for example, in cerebrotendinous xanthomatosis.[4,5] The protein constituents of lipoproteins, the apoproteins, are responsible for emulsifying or solubilizing the attached lipids for transport in the plasma and 12 apoproteins have been identified. Type IB includes patients with alterations in lipoprotein or apoprotein structure. Our patient's clinical features did not coincide with those of cerebrotendinous xanthomatosis but, since the precise structure of the apoproteins was not identified, an apoprotein abnormality remains a possibility. Type II represents a group in which diffuse-plane xanthomatous lesions are associated with an underlying lymphoproliferative disease with paraproteinaemia, such as multiple myeloma, Waldenstrom's macroglobulinaemia, cryoglobulinaemia, lymphoma, and benign monoclonal gammopathy.[6]

Type III normocholesterolaemic xanthomatosis comprises a heterogeneous group of patients in which there is neither evidence of lipoprotein abnormalities nor associated underlying systemic disease. This includes xanthoma disseminatum and, to date, there has been one previous case report of 'normolipaemic' subcutaneous xanthomatosis.[3] A 62-year-old man presented with multiple subcutaneous xanthomatous nodules associated with vocal cord xanthomata and diabetes mellitus. The serum lipid profile was reported to be 'essentially normal', apart from a slight decrease in total cholesterol and high-density lipoprotein levels.

In our patient the development of slightly increased serum levels of total and VLDL triglycerides in the presence of normal cholesterol levels did not constitute a recognized type of hyperlipoproteinaemia.[1] However, the decreased HDL cholesterol level might have been of pathogenic importance, since low levels of total HDL have been reported in patients with xanthelasmata, tuberous xanthomata and, as shown at post-mortem examination in this case, coronary atherosclerosis.[7] A normal level of HDL appears to serve in removing cholesterol from the tissues, providing a mechanism for protection against atherosclerosis and xanthoma formation.

In conclusion, this unusual patient presented with multiple subcutaneous xanthomatoses in the absence of a recognized type of hyperlipoproteinaemia or histiocytic syndrome. He showed no evidence of a lymphoproliferative disorder or diabetes mellitus. The carcinoma of the rectum appears to have been a coincidental finding but the post-mortem evidence of coronary atherosclerosis might have been related both to the decreased level of HDL cholesterol and to the subcutaneous xanthomatosis.

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Urticarial vasculitis associated with a monoclonal IgM gammopathy: Schnitzler's syndrome

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SUMMARY

We describe two cases of Schnitzler's syndrome presenting with urticarial vasculitis, in which there was a monoclonal IgM of kappa light-chain isotype. Skin histology showed a perivascular leucocytic infiltrate and leucocytoclasis. One patient had an abnormal complement profile and positive rheumatoid factor while the other had antibodies to unidentified soluble nuclear antigens, anti-cytoplasmic antibodies and circulating immune complexes. In addition, in the first case deposits of IgM, C3 and, to a lesser degree, of IgG were observed in the walls of small blood vessels.

Since its initial description by Schnitzler in 1974, at least 16 cases of chronic urticaria associated with a monoclonal IgM globulin have been reported with varying combinations of extracutaneous manifestations, including fever, weight loss, arthralgias and bone pain.[1–8] Skin biopsies often show a mild to severe leucocytoclastic vasculitis. This syndrome is a distinct clinical entity within a spectrum of disorders that include chronic urticaria, hypocomplementaemic vasculitis, autoimmune and viral diseases that may show an urticarial vasculitis with damage to the skin vessels.[9] We report two further cases of Schnitzler's syndrome of chronic urticaria associated with a monoclonal gammopathy of IgM type.

CASE REPORTS

Case 1

A 70-year-old female was admitted to hospital in December 1987 with a 4-year history of recurrent episodes of non-pruritic urticarial and purpuric lesions, principally involving the thighs, buttocks and trunk. At that time she had other symptoms of fatigue, weight loss,

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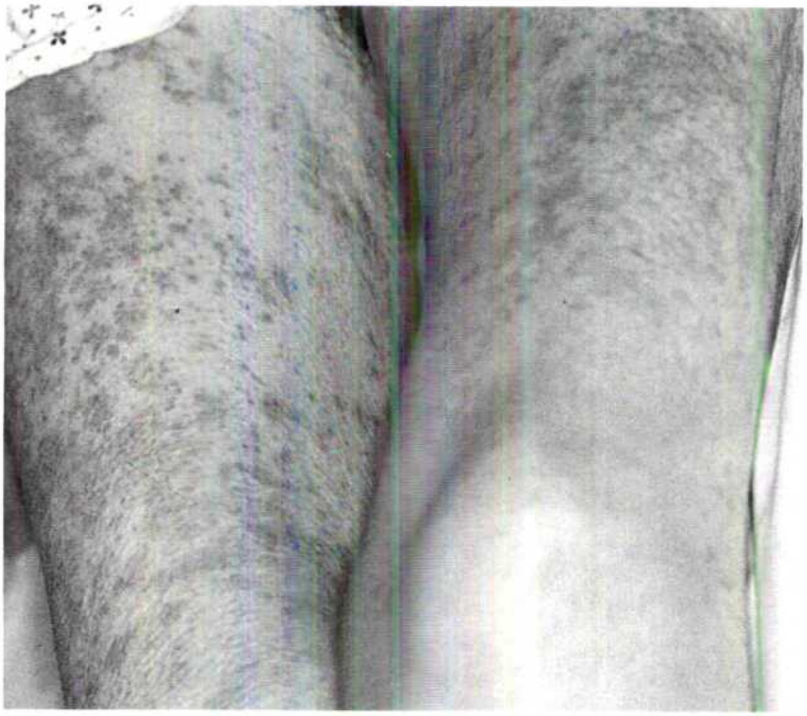

FIGURE 1. Case 1. Weals over the anterior thighs resolving with palpable purpuric lesions.

arthralgias and deep bone pain in the legs and forearms. The patient had typical urticarial weals on the lower limbs, trunk and forearms that persisted for several hours, leaving palpable purpuric lesions (Fig. 1). The remainder of the physical examination was normal, except for tenderness to pressure over the tibia and an effusion of the right ankle.

A skin biopsy of an urticarial lesion showed moderate dermal oedema with an obvious leucocytoclastic vasculitis and a dense perivascular infiltrate that consisted predominantly of neutrophils and with fibrin deposits in the vessel walls (Fig. 2). Direct immunofluorescence (IF) of perilesional skin showed deposits of IgM and C3 and to a lesser extent, IgG in the walls of small vessels.

There was a normochromic normocytic anaemia of 110 g/l and an erythrocyte sedimentation rate of 32 mm/h. The white cell and platelet counts, electrolytes, liver function tests, serum creatinine and urine analysis were all within normal limits. Rheumatoid factors were present (97 IU/ml, normal range: < 60 IU/ml), but there were no cryoglobulins and antinuclear antibodies. Serological tests for syphilis and hepatitis B virus were negative. Analysis of a stool specimen did not show any parasites or ova. Paper electrophoresis of the serum proteins was normal, but immunoelectrophoresis showed an M component of IgM-κ of 2·7 g/l. Free light chains were detected in the urine. The serum IgG and IgA levels were normal.

Abdominal ultrasound and skeletal scintigraphy as well as bone-marrow aspirate and biopsy provided no evidence for an abnormal lymphoid proliferation. X-rays of the bones showed a diffuse osteoporosis without lytic lesions. In multiple individual samples drawn at different times, sequential titration of haemolytic complement by sensitized sheep red cells revealed

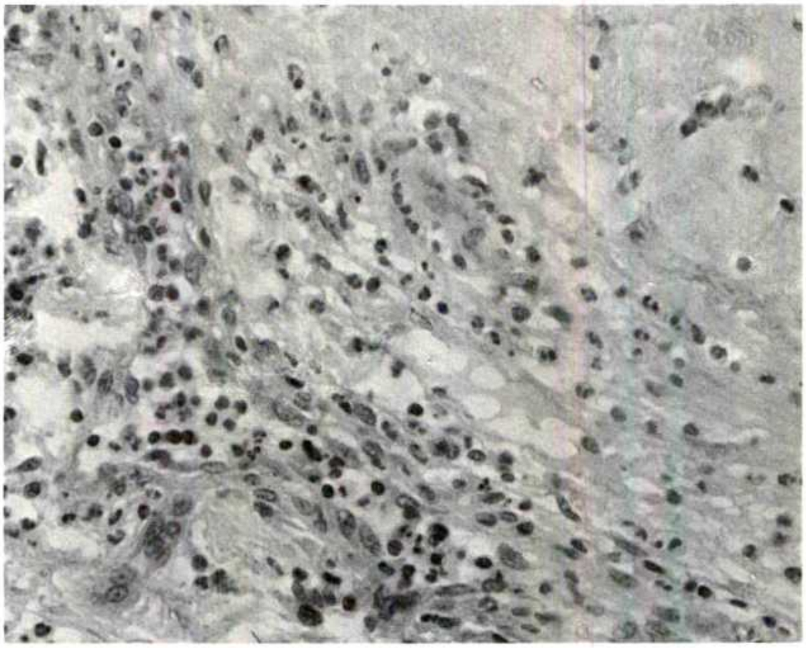

FIGURE 2. Case 1. Leucocytoclastic vasculitis; fibrin deposits around and in capillary walls; perivascular infiltrate predominantly of neutrophils and nuclear dust (haematoxylin and eosin, × 162).

activities of 50–70% of those of a pool of normal donors. This was associated with decreased levels of C4 between 3·90 mg/dl and 5·82 mg/dl (pool of normal donors: 13–40 mg/dl) and slightly reduced to normal C3 levels, indicating an activation of the classical complement pathway. Concentrations of C1q were reduced (33%; 95% prediction intervals of healthy adults: 60–135%) and C1q-precipitins were not detectable.

Treatment with colchicine 1 mg daily was started in December 1987 with considerable improvement and in the past 16 months this has kept her asymptomatic.

Case 2

A 48-year-old male was first admitted to hospital in June 1987 with a 6-month history of recurrent, almost daily episodes of a non-pruritic urticarial rash, involving the trunk and extremities. The urticarial weals were up to 2 cm in diameter and persisted for 24–36 h. The eruption was accompanied by an intermittent fever, fatigue and moderate weight loss. He also had axillary and inguinal lymphadenopathy. A skin biopsy showed a mild leucocytoclastic vasculitis in the upper dermis. The ESR was increased to 45 mm/h. Full blood count, electrolytes, hepatic enzymes, serum creatinine, and analysis of the urine were normal. Cryoglobulins and rheumatoid factors were not detected. When the patient's serum was tested by indirect immunofluorescence at 1:20 dilution,[10] antinuclear antibodies could not be detected, but with a double immunodiffusion assay[10] antibodies directed to unidentified soluble nuclear antigen were found. Antibodies to double- and single-stranded DNA, SS-A/Ro, SS-B and Sm were not present. The serological tests for hepatitis B virus, cytomegalovirus, human immunodeficiency virus and toxoplasmosis were negative. Analysis of a stool specimen was normal.

Serum complement haemolytic activity (64 CH50; normal values of a pool of blood donors 70 ± 10 CH50), C4 (68·1 mg/dl; normal range 70–145 mg/dl) and C3 (13·9 mg/dl; normal values 13–40 mg/dl) levels were in the low normal range. Immune complexes were detected using a polyethylene glycol 6000 precipitation test with subsequent demonstration of fixed C1q. Ultrasound and CT scanning of the abdomen gave normal results. No abnormal lymphoid proliferation was observed in biopsies of lymph node and bone marrow.

Treatment with colchicine 1 mg daily produced no improvement and subsequently, dapsone 100 mg daily was given, but the patient continued to have episodes of urticaria accompanied by muscle pain and arthralgia. In November 1987 the patient was given 50 mg prednisone a day and this resulted in the control of the skin rash and symptoms. On reducing the dose of systemic steroids to < 10 mg daily the urticarial lesions reappeared. In March 1989 a further skin biopsy showed a moderate perivascular infiltrate of lymphocytes and histiocytes with a leucocytoclastic vasculitis. Direct immunofluorescence showed deposits of IgM at the dermo-epidermal junction with C3 in the walls of small vessels. The ESR was 90 mm/h and there were increased levels of α-2 globulin (9·2 g/l) and gamma globulin (23·8 g/l). Immunoelectrophoresis of the serum proteins showed a monoclonal IgM-κ of 13 g/l. The level of IgA was decreased. Skeletal investigations that included X-rays and scintigraphy were normal. Anti-cytoplasmic antibodies (ACPA) in a titre of 1 : 16 were found.[11] The complement haemolytic activity, C4 and C3 levels were in the lower normal range and immune complexes were detected.

DISCUSSION

The main feature of Schnitzler's syndrome is the association of chronic urticaria with a monoclonal IgM globulin, usually of kappa light isotype, and clinical features for Waldenström's macroglobulinaemia are absent.[1–7] Our patients had also general symptoms of fatigue, intermittent fever, weight loss, arthralgias and muscle and/or bone pain. Apart from axillary and inguinal lymphadenopathy in Case 2, no other extracutaneous signs were present. In a recent review of 15 cases, Janier *et al.*[7] found lymph-node enlargement in about 50% of the cases, hepatomegaly in less than 30% and splenomegaly in just one out of the 15 patients. In addition, X-rays of the bones may show dense lesions and a positive radionucleotide scan, but no specific abnormality is found at the biopsy.[6,7,12] On skin biopsy there is a perivascular inflammatory infiltrate in the upper dermis consisting of lymphocytes or neutrophils and in half of the patients a moderate to severe leucocytoclastic vasculitis.[2,7]

In our first case unusual findings were the activation of the classical complement pathway and the presence of rheumatoid factors. In our second case there were circulating immune complexes with a low normal level of complement. Hypocomplementaemia may occur in patients with cryoglobulinaemia and lymphoproliferative disorders, as a result of the activation of C1 by abnormal immunoglobulins.[13] In our second patient, nuclear antigen antibodies were detected, but they could not be identified. It remains unclear whether these antibodies play any role in the pathogenesis of this condition. This patient also had positive ACPA which, though thought to be a specific marker for Wegener's granulomatosis,[11] have also been found in other vasculitic disorders.[11]

Immunofluorescence studies showed deposits of C3, IgM and, to a lesser degree, of IgG in the walls of small vessels in our first patient. In none of 10 patients with Schnitzler's syndrome in which immunofluorescence studies were performed[7] were deposits of IgM found in the vessels walls, but in three cases deposits were located in the upper dermis or at the basement membrane zone as occurred in our second patient.

Although the long-term course of the disease is usually benign, the patients should be closely followed up because they may develop a lymphoproliferative malignancy.

The exact role of the monoclonal IgM in the pathogenesis of urticaria is unknown. The presence of abnormal deposits of IgM and C3 in the walls of small vessels as detected in our first patient strongly suggests direct involvement in the development of the lesions. As previously observed,[4] monoclonal IgM may be deposited in the skin and may form circulating immune complexes which, once deposited, could in turn activate complement cascade and enhance tissue damage.[14] Further immune complexes could be formed *in situ* with the combination of the monoclonal IgM with an unidentified antigen.[14] It has recently been claimed that the monoclonal IgM globulin reacts with interleukin-1.[8]

The response of our first patient to colchicine was unexpected. In previous reports the skin lesions and extracutaneous symptoms did not respond to colchicine or to a variety of other treatments that have included H_1 and H_2 receptor antihistamines, chloroquine, dapsone, cyclophosphamide, chlorambucil and azathioprine.[2,6,7] Only high doses of corticosteroids and nonsteroidal anti-inflammatory drugs have been shown to have an effect on urticaria and bone pain.[2,6,7]

Our observations suggest that a subset of patients with chronic urticarial vasculitis associated with IgM paraproteinaemia may present with previously unreported serological and immunopathological findings, including evidence of complement activation, and the presence of nuclear antigen and anti-cytoplasmic antibodies.

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Toxic pustuloderma: a self-limiting eruption

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SUMMARY

Three patients are described who developed numerous pinhead sized pustules within areas of a widespread toxic erythema. The eruption was precipitated by food poisoning in one patient, a suspected, but blood-culture-negative septicaemia in another and in the third patient, by a cephalosporin. This self-limiting syndrome consists of fever, a pustular and erythematous eruption, a neutrophil leucocytosis, subcorneal and spongiform pustules but without a history of psoriasis. We believe that this entity of toxic pustuloderma represents a severe form of toxic erythema.

CASE REPORTS

Patient 1

A 41-year-old Portuguese housewife presented in February 1985 with a sore and itchy rash which first appeared under both breasts and gradually spread to involve the trunk and limbs. She was convinced that the rash was precipitated by eating fish 12 hours earlier that smelt bad. The patient denied taking any medication, had no significant past medical history and her general health was good. There was no family history of any skin disease.

On examination the patient was pyrexial at 38·2°C. There were confluent areas of livid erythema on the chest and back extending into both axillae and down the medial surfaces of both arms as far as the antecubital fossae. Similar lesions were present on the abdomen extending inferiorly down both legs and involving the natal cleft. There was slight scaling of the scalp. Within the areas of erythema on the trunk and flexures there were numerous tiny pinhead sized pustles (Fig. 1).

Investigations revealed a white blood cell count of $10{\cdot}5 \times 10^9$/l with 75% neutrophils and 20% lymphocytes, a negative antinuclear antibody and cultures of swabs taken from the pustular lesions, blood cultures and a throat swab were sterile. Serological tests for cytomegalovirus, adenovirus, Epstein–Barr virus, hepatitis A and B were negative.

The patient was treated with potassium permanganate baths, a topical preparation of 0·05% clobetasone butyrate containing chlortetracycline and nystatin (Trimovate cream®) and there was complete resolution of the lesions within 6 days. There has been no recurrence of any rash over a 4-year follow-up period.

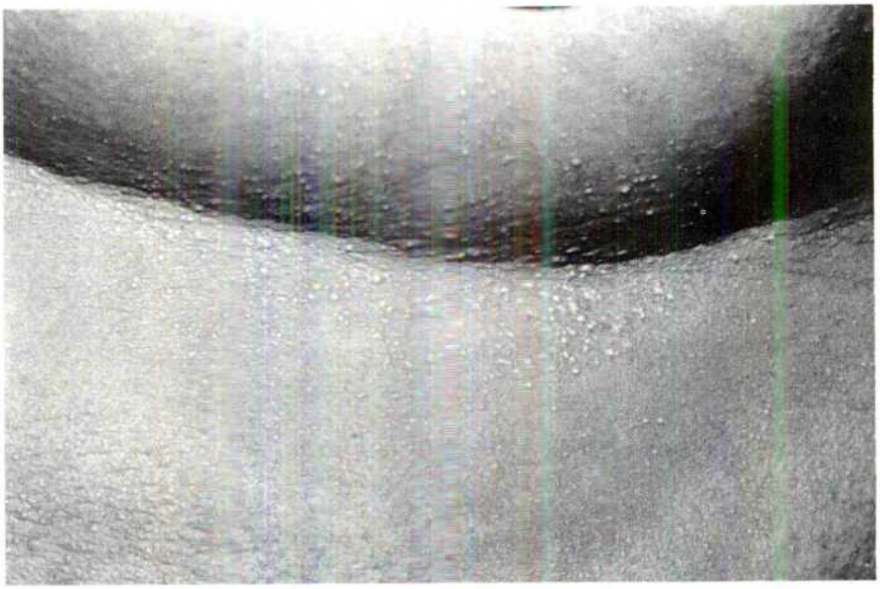

FIGURE 1. Patient 1. Numerous superficial pinhead sized pustules superimposed on a background of erythema below the right breast.

Patient 2

A 48-year-old Black lorry driver sustained mixed thickness burns to the hands and arms in a road traffic accident. Because of smoke inhalation and the development of the respiratory distress syndrome he required artificial mechanical ventilation. In addition, because of a suspected, but blood-culture-negative septicaemia, intravenous gentamycin and intravenous clindamycin were administered. On the ninth day of admission whilst still pyrexial at 39·6°C he developed a diffuse erythema involving the trunk and limbs. Superimposed on this erythema were myriads of pinhead sized pustules. Cefuroxime was substituted for the clindamycin and gentamycin and within 48 h the patient became apyrexial and the erythema faded. Without any topical treatment the pustules dried up and this was followed by mild desquamation. The patient died on day 20 with progressively worsening respiratory failure and disseminated intravascular coagulation.

Investigations at the time of the pustulation showed a white blood cell count of $25{\cdot}7 \times 10^9/l$ with 94·8% neutrophils. Swabs of pustular lesions and repeated blood cultures were sterile but culture of tracheal aspirate, nasal swabs and urethral swabs grew *Pseudomonas aeruginosa*.

Patient 3

A 55-year-old man underwent a routine cholecystectomy and exploration of the common bile duct for gall stones in August 1988 and post-operatively developed peritonitis. A laparotomy revealed faecal peritonitis but no obvious site of dehiscence. Within 24 h he became shocked, septicaemic, required ventilation and haemofiltration for acute renal failure. Subsequently he has had recurrent septicaemic episodes, a left subphrenic abscess and multiple chest infections. During one of these suspected, but blood-culture-negative septicaemias, cultures of sputum grew *Candida albicans*, Gram negative rods. *Pseudomonas aeruginosa* and *Flavobacterium* spp. He was treated with intravenous metronidazole, ceftazidine, vancomycin and amphotericin.

Four days later the patient developed a diffuse erythematous eruption on the trunk, limbs, face and scalp and within these areas there were numerous pinhead sized pustules. All the antibiotics were stopped, Trimovate cream® was applied topically and over the next 5 days the erythema and pustulation resolved with subsequent desquamation of the affected skin. The white blood cell count at this time was $24{\cdot}1 \times 10^9$/l with 87·5% neutrophils. Swabs of pustular lesions were sterile.

Two months later whilst still being ventilated sputum cultures grew Gram negative rods, *Pseudomonas aeruginosa* and *Flavobacterium* spp. but the patient did not develop any cutaneous eruption. However, in December 1988 the patient became pyrexial at 39°C, blood cultures grew *Streptococcus faecalis* and he was treated with intravenous benzyl penicillin and cefuroxime. Forty-eight hours later he developed a generalized erythroderma, and soon afterwards numerous pinhead sized pustules on the trunk, limbs and in the flexures. The white blood cell count was $30{\cdot}1 \times 10^9$/l with 73·4% neutrophils and 21·3% lymphocytes. The antibiotics were stopped, Trimovate cream® was applied topically to all the affected areas and following a period of desquamation the skin returned to normal within 2 weeks.

Histopathology

Skin biopsies of affected areas from the three patients showed similar epidermal changes and in all there were subcorneal pustules (Fig. 2), spongiform pustules of Kogoj (Fig. 3) and spongiosis and in two there were follicular pustules. The biopsy from patient 2 showed areas of vacuolation of cells in the stratum spinosum and loss of the stratum granulosum. There was capillary dilatation and oedema of the papillary dermis in all the biopsies, that was particularly marked in two of them. A variably intense, predominantly lymphocytic perivascular infiltrate in the mid and papillary dermis was observed in all biopsies

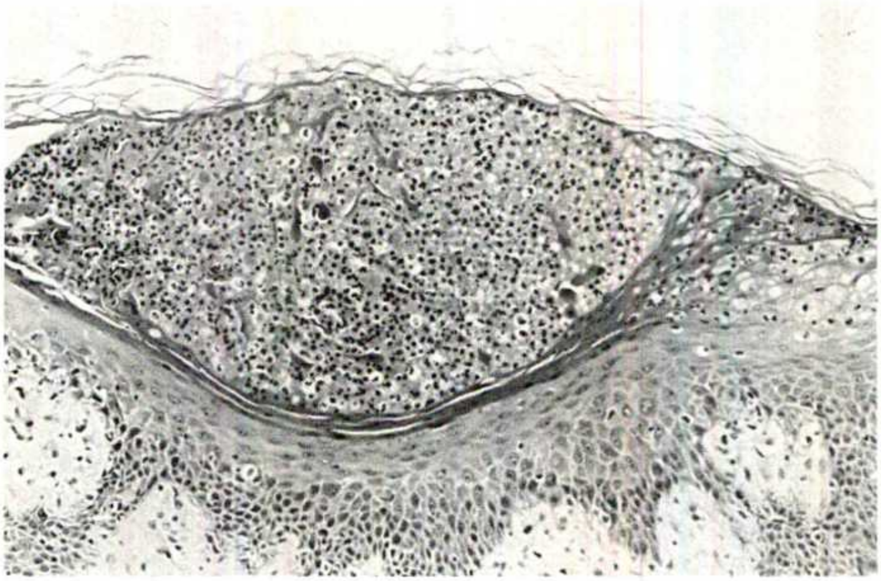

FIGURE 2. Subcorneal pustule containing large number of polymorphonuclear leucocytes. Haematoxylin & eosin × 70.

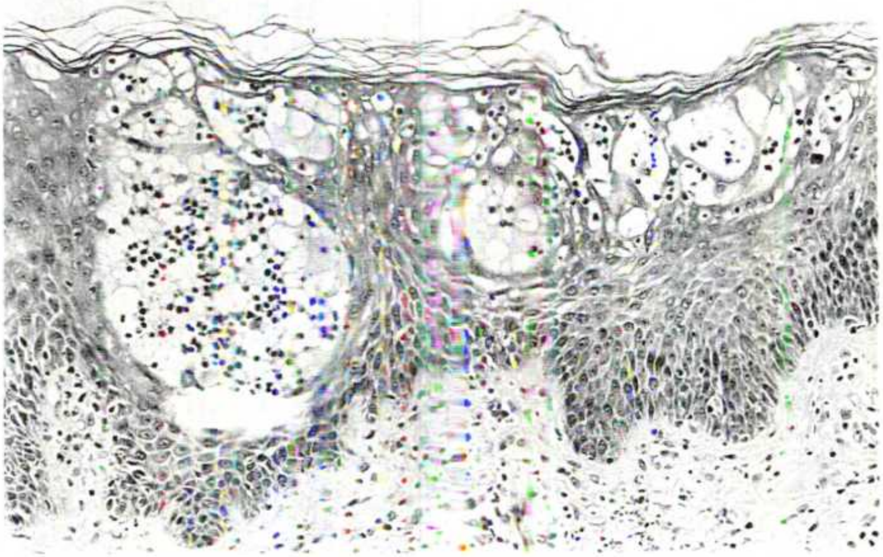

FIGURE 3. Spongiosis and spongiform pustules of Kogoj. Haematoxylin & eosin × 70.

DISCUSSION

There are many diseases which present with sterile epidermal neutrophilic pustulation[1] and these include pustular psoriasis,[2] palmoplantar pustulosis,[2] subcorneal pustular dermatosis,[3] pustular bacterid,[4] pustular necrotizing angiitis,[5] erythema multiforme,[6] Sweet's syndrome[7] and halogen exposure.[8] Subcorneal pustular dermatosis can be distinguished by the fact that the latter condition is more chronic and the pustules mainly occur in the groins, axillae, submammary areas and have a transient surrounding erythematous flare rather than being based on extensive areas of erythema.[3] Similarly pustular necrotizing angiitis can easily be distinguished since these patients develop large pustules (up to 5 cm in diameter) and rupture of the pustules reveals necrotic ulcers which heal to leave depressed scars.[5] The morphological features of Sweet's syndrome do not resemble toxic pustuloderma since pustules when they do occur usually arise from within the well-defined painful plaques or nodular infiltrates and generally this condition lasts for weeks or months unless corticosteroids are administered.[7]

There are other dermatoses that may be confused with toxic pustuloderma. Four clinical patterns of pustular psoriasis, namely the Zumbusch, annular, localized and exanthematic types have been described.[2] The exanthematic type appears to closely resemble toxic pustuloderma in that the patients had not previously suffered from psoriasis and the eruption followed infections or administration of drugs. The episodes were also short lived and completely resolved within a few weeks.

Andrews and Machacek described a recalcitrant pustular eruption symmetrically located on the palms and soles which was thought to be related to a focal infection usually in the tonsils but also in tooth abscesses.[4] Removal of the septic focus did not cure this eruption[9] and there has been debate as to whether these eruptions represent a manifestation of psoriasis or a bacterid. The description by Tan[10] of an acute generalized pustular bacterid with vasulitis suggests the existence of a bacterid and possibly the patient described by Sneddon who had subcorneal pustules in association with target lesions and severe musocal involvement of the Stevens–Johnson type had a combination of a bacterid and erythema multiforme.[6]

Patients having similar clinical features to those described in this paper have previously been described in the literature under many different titles: erythemo-squamous and pustular spongiform eruption,[11] generalized pustular drug rash,[12] generalized pustular toxic erythema,[13] pustulose exanthématique aiguë généralisée,[14,15] acute generalized exanthematous pustular dermatitis[16] and toxic pustuloderma.[17] The patients have episodes of a pustular and erythematous eruption associated with fever, epidermal spongiform pustules, a peripheral blood leucocytosis and all have had an absence of antecedent psoriasis. The lesions resolve often without any treatment within 1–3 weeks. There may also be evidence of a vasculitis or vascular deposition of IgM and C3.

The eruption may be precipitated by either infections or drugs and although in many cases there is good circumstantial evidence that drugs are causative there are other patients[13,15] (Patient 1) who have not received any previous medication. The drugs most frequently incriminated are antimicrobials (penicillin, ampicillin, metronidazole, trimethoprim and sulphamethoxazole), antimalarials (pyrimethamine), diuretics (frusemide) although other medications including piperazine, carbamazepine and chloramphenicol have been implicated.

Recently there have been three reports in the American literature of pustular eruptions and generalized erythema following administration of cephalosporins.[18–20] In our third patient there was circumstantial evidence that an antimicrobial agent had caused the toxic pustuloderma on the first occasion. However, the only antibiotic that was given on the first occasion and again before the second episode of toxic pustuloderma was a cephalosporin. We consider therefore that this is the first of such reports from the U.K. although data extracted from the Committee on Safety of Medicines records over the period July 1963–November 1988 show that of the total of 177 reports of adverse reactions to cefuroxime and of the 67 to ceftazidime, there was one case of a pustular rash with cefuroxime and none with ceftazidine (Committee on Safety of Medicines, personal communication). The case of our third patient has therefore been reported to the Committee on Safety of Medicines.

It is clear that the erythematous and pustular eruption described above is not new and has been recognized by other dermatologists in the past. We suggest that the name toxic pustuloderma, coined by Staughton and colleagues in 1984, should be retained and adopted for such a dermatosis.

ACKNOWLEDGMENT

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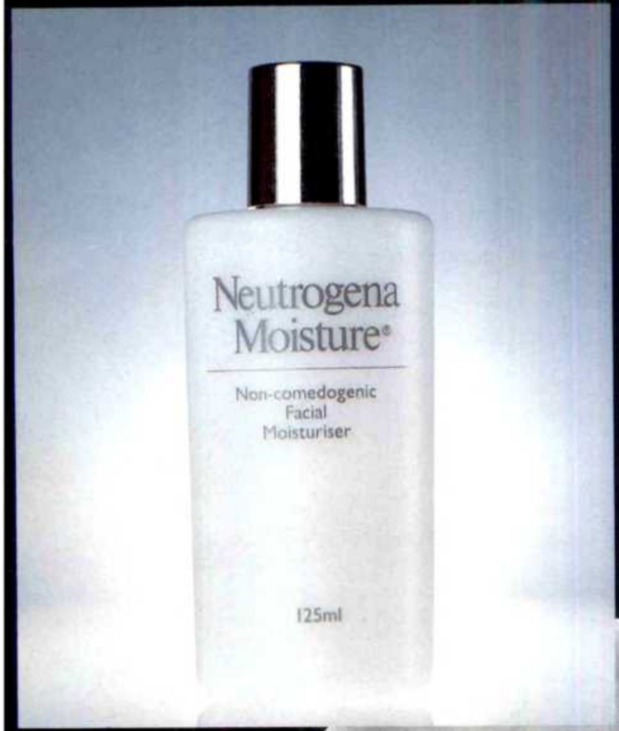

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Correspondence

Obliterative bronchiolitis and alveolitis associated with sulphamethoxypyridazine (Lederkyn) therapy for linear IgA disease of adults

SIR, We were interested to read the report by McFadden *et al.*[1] on the treatment of dermatitis herpetiformis, linear IgA disease and cicatricial pemphigoid with sulphamethoxypyridazine and a case of alveolitis while on this drug. We have reported previously a patient in the thoracic literature whose lung function became severely compromised by bronchiolitis obliterans and alveolitis while taking sulphamethoxypyridazine for adult linear IgA disease.[2] We consider it important to bring this serious side-effect of sulphamethoxypyridazine to the attention of dermatologists who are the principal users of this drug.

A 49-year-old female developed an extensive pruritic blistering eruption associated with conjunctival, nasal and oral mucosal lesions. Direct immunofluorescence demonstrated a bright linear band of IgA at the basement membrane zone, and indirect immunofluorescence a low titre IgA antibody that localized to the dermal side of saline split skin.[3] Her eruption responded well to dapsone, but this was stopped because of profound malaise, fever, painful lymphadenopathy and abnormal liver function tests. It was considered that this represented the dapsone hypersensitivity syndrome.[4] Because of the recurrence of blistering she was then started on sulphamethoxypyridazine in an initial dosage of 250 mg daily and later up to 1·5 g daily. This was combined with prednisolone 15 mg daily. Two months after starting sulphamethoxypyridazine she complained of increasing breathlessness on exertion. Respiratory examination and chest X-rays were unremarkable on several occasions. Over the next 3 months her dyspnoea became progressively worse and she was admitted for assessment. Her chest X-ray at that time showed diffuse bilateral interstitial shadowing. Lung function tests were impaired with a restrictive pattern (FEV_1 0·5 l and FVC 0·8 l; predicted FEV_1 1·9–3·1 l, FVC 2·2–3·6 l) and her arterial oxygen partial pressure (PaO_2) was grossly reduced at 6·2 kPa (normal range 10·0–13·3 kPa). Open lung biopsy performed to exclude lymphoma or opportunistic infection showed extensive focal interstitial fibrosis, with minor lymphocytic infiltration and the presence of type 2 pneumatocytes and an obliterative bronchiolitis.

The diagnosis was thought to be drug-induced lung disease, and the sulphamethoxypyridazine was stopped. Prednisolone was increased to 60 mg daily and within 5 weeks her chest X-ray shadowing had cleared and the PaO_2 had normalized to 13·6 kPa and the FVC improved to over 2 l. Six months later however, her exercise tolerance is still limited by breathlessness.

We consider that the time course and pathology of the lung disease strongly implicates sulphamethoxypyridazine as being the responsible agent. Bronchiolitis obliterans and alveolitis are well recognized as occurring in association with sulphasalazine therapy for inflammatory bowel disease[5–7] and sulphasalazine has been associated with an acute hypersensitivity pneumonitis soon after starting therapy.[8] It is important to consider this adverse reaction in patients on sulphamethoxypyridazine who develop unexplained breathlessness.

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Atopy: a prostaglandin E precursor- and receptor-dependent defect in T-cell maturation and function?

SIR, In 1937, Brown and Hansen reported decreased serum levels of arachidonic acid in patients with atopic dermatitis and suggested that a disturbance in the metabolism of long-chain unsaturated fatty acids might be one of the factors at fault in atopic dermatitis.[1] Later investigations confirmed that plasma phospholipids of patients with atopic dermatitis exhibit decreased levels of γ-linolenic acid (GLA), dihomo-γ-linolenic acid (DGLA) and arachidonic acid, whereas the levels of linoleic acid are higher than normal, suggesting defective functioning of the enzyme δ-6-desaturase.[2] Deficiencies in DGLA and arachidonic acid might result in impaired formation of the biologically important prostaglandins E_1 and E_2 (PGE_1 and PGE_2) (Fig. 1). In fact, in comparison with normal non-atopic skin significantly decreased levels of PGE_2 have been observed in the uninvolved skin of patients with atopic dermatitis.[3] A correlation has been shown between increased levels of linoleic acid in umbilical cord serum lecithin and elevated cord serum immunoglobulin E (IgE).[4]

Normal human lymphocytes exhibit high-affinity binding sites for PGE_1 and PGE_2. However, atopic T lymphocytes showed a decreased sensitivity to PGE_2 which could be explained by a significant reduction of PGE_2 receptors on atopic T lymphocytes.[5] PGEs exert their biological effects by an induction of the adenylate cyclase activity resulting in an intracellular increase of cyclic adenosine monophosphate (cAMP) (Fig. 1).

From *in vitro* experiments it is concluded that cAMP is the intracellular mediator for regular differentiation of prothymocytes to thymocytes.[6] The cAMP inducer PGE_1 is present in the thymus and reveals thymus-hormone-like activities in promoting T-lymphocyte maturation and function.[7] Human thymic epithelial cells produce substantial amounts of PGE_2.[8] In mice, PGE_2 has been demonstrated to induce the appearance of cell surface markers in immature T lymphocytes. Thymic hormones lead to further differentiation of thymocytes through enhanced release of PGE_2.[9] These observations underline the important role of PGEs for maturation and differentiation of T lymphocytes in the thymus.

To examine the role of suppressor T cells in atopic persons, several investigators have studied the effects of normal *in vitro* IgE production by cultured peripheral blood mononuclear cells from patients with severe atopic dermatitis or hyper-IgE syndrome. Patients with the hyper-IgE syndrome, the maximal variant of impaired downregulation of IgE synthesis, exhibited the most significant deficiency of suppressor T cells. The spontaneous IgE synthesis by cultured peripheral blood mononuclear cells of children with the hyper-IgE syndrome could be suppressed by the addition of suppressor T cells from non-atopic parents.

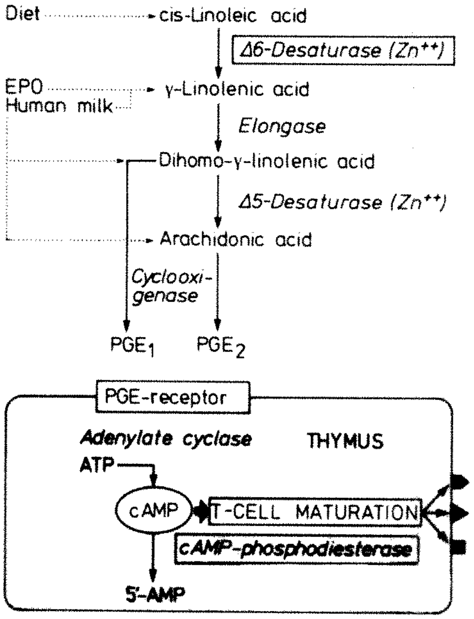

FIGURE 1. The role of long-chain ω-6-fatty acids and E-prostaglandins in normal T lymphocyte maturation. EPO, evening primrose oil; PGE, prostaglandin E; cAMP, cyclic adenosine monophosphate.

Human T lymphocytes exhibiting high affinity binding sites for PGE_2 had a strong suppressor effect on pokeweed mitogen-driven B-cell maturation into immunoglobulin-containing cells.[10] Thirty-three per cent of these PGE2-binding T lymphocytes expressed the suppressor T-cell marker CD8. The question arises whether the PGE_2-receptor deficiency on atopic T lymphocytes is involved in insufficient maturation and function of PGE_2-binding suppressor T cells of atopic individuals. Support for this suggestion comes from mice foetal thymic-organ cultures which synthesized PGE_2 and were found to be capable of metabolizing exogeneous arachidonic acid to PGE_2, whereas the addition of the cyclooxygenase inhibitor indomethacin inhibited the expression of Lyt-2, the surface marker characterizing mouse suppressor T cells.[11] The addition of 10^{-6}–10^{-5}M PGE_1 or PGE_2 to peripheral blood mononuclear cells of patients with atopic dermatitis resulted in a significant inhibition of spontaneous *in vitro* IgE synthesis (Melnik *et al.*, unpublished observation). Both the deficiency of the long-chain ω-6-fatty acids and the PGE_2-receptor deficiency of atopic T lymphocytes during early thymic T-cell maturation might cause the development of the atopic immune system. Indeed, *in vivo* studies with anti-PGE antibodies suggest that PGEs are important mediators for the early phase of cell-mediated immunity.[12]

Further evidence for the role of essential ω-6-fatty acids and PGEs for T-cell maturation and regulation of suppressor T-cell function is provided by the observation that prolonged breast-feeding protects against the development of atopy later in life. Human colostrum and mature human milk, in contrast to cow milk-based formulae, are rich in GLA, DGLA and arachidonic acid. The finding that milk of atopic mothers contains reduced levels of δ-6-desaturase products is not surprising.[13] These recent insights offer a novel approach for the prevention of atopy by administration of sufficient amounts of GLA to the atopic pregnant and nursing woman and her baby during the first months of postpartal life. GLA in the form of evening primrose oil has already been shown to improve atopic dermatitis. Supplementation of GLA during early T-cell maturation and differentiation in the thymus might compensate for the missing PGE-stimuli due to the δ-6-desaturase-dependent deficiency of PGE-precursor long-chain fatty acids and the PGE_2-receptor deficiency on atopic T lymphocytes.[14]

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ADDENDUM IN PROOF

Pène *et al.*[15,16] recently demonstrated that physiologically relevant concentrations of PGE_2 significantly suppressed interleukin-4-induced IgE production of peripheral blood mononuclear cells from healthy non-atopic donors.

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Skin diseases that may mimic child abuse

SIR, The subject of child abuse is never far from the headlines. It is important to consider skin diseases which may mimic abuse. The topic has recently been aired with particular reference to 'burns and scalds'.[1] We report a dramatic example of this—dystrophic epidermolysis bullosa masquerading as burns. In its severe form this disease is an aggressive, often very disabling, disease characterized by extensive blistering and subsequent scarring of the integument and upper gastrointestinal tract. In its milder forms, however, trauma-induced blisters may be confined to the acral bony prominences, and occasionally the only physical sign is an isolated dystrophic nail. Recently two siblings, aged 3 and 4 years, were referred as a prelude to being taken into care because of the suspicion, by two doctors, that they had received cigarette burns to their hands and legs. This was despite their mother's protestations that she too had suffered with trauma-induced blisters as a child. The diagnosis of child abuse should never rest on a single finding and the social background had to be considered. On this occasion examination of the family confirmed that all three had dominant dystrophic epidermolysis bullosa. Both children demonstrated fresh erosions over the knees, elbows, knuckles, ankles and forehead, with associated scarring and milium formation. Their mother had thin scars over her knees and elbows, together with 'albopapuloid' lesions on her back.

There are more familiar dermatological entities, such as lichen sclerosus et atrophicus of the genitalia and Mongolian blue spots, that may be mistaken for child abuse by the inexperienced. Dystrophic epidermolysis bullosa, particularly when the disease is mildly expressed, is a further condition.

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Is benzoyl peroxide carcinogenic?

SIR, Benzoyl peroxide was one of the first substances found to have a clearcut effect on acne vulgaris on topical application. It was introduced in Canada in the early 1960s[1] and is now widely used in various preparations. Experiments on rodents have shown that benzoyl peroxide acts as a promoter for skin cancer,[2,3] and because of this there has been some concern. In 1987, the Canadian Health Protection Branch recommended that benzoyl peroxide be placed on a special 'Schedule F' and that the sale of cosmetics containing this compound should not be permitted. An information letter to all concerned health professionals was to be prepared and a retrospective case-control study given consideration.[4]

So far no increase in skin malignancies after the clinical use of benzoyl peroxide has been reported. A possible relationship between the use of the compound and the occurrence of malignant melanoma has been looked at in two case-control studies, both with negative results.[5,6] In Sweden products containing benzoyl peroxide were introduced in 1977 and the sales have increased from 341 kg in 1981 to 1103 kg in 1984. It is mainly used by the young in the age group 10–29 years. The number of skin malignancies on the face has been determined from data claimed from the Swedish Cancer Registry and these are shown in Figure 1. During the 8–9 years since the introduction of benzoyl peroxide there has been no change in the frequency of skin malignancies on the face. However, since the average latent period for skin carcinogenesis is of the order of 15–25 years,[7] this requires further follow-up and should an increase occur then case-control studies would be indicated.

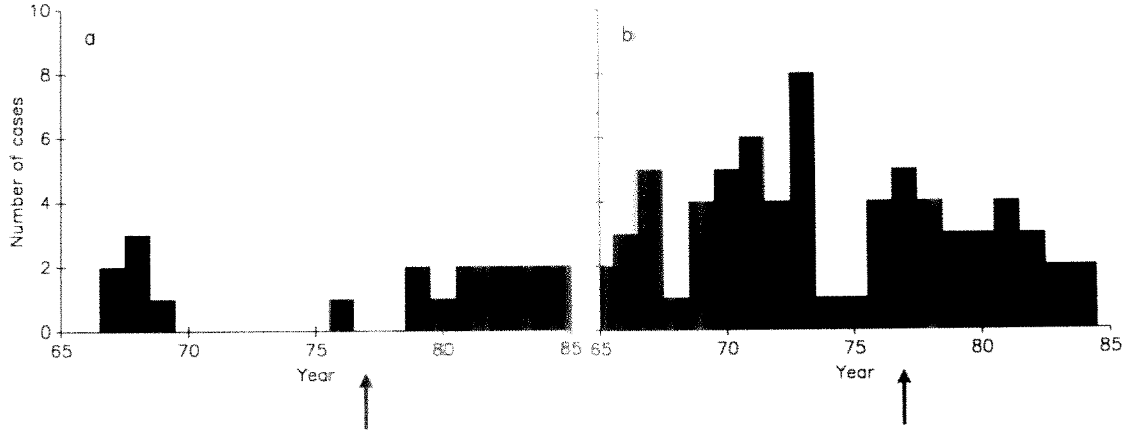

FIGURE 1. Number of new cases of skin malignancies on face, scalp and neck reported 1965–1985 to the Swedish Cancer Registry for the age group 10–29 years. (a) Non-melanoma skin cancers (basal cell cancers not included); (b) malignant melanoma. The arrow indicates introduction of benzoyl peroxide.

Recently it has been shown that ornithine decarboxylase, that is considered to be necessary for tumour promotion, can be induced by benzoyl peroxide under certain experimental conditions.[8] However, the carcinogenic properties of benzoyl peroxide could not be confirmed in another study.[9] Thus, the question of carcinogenic potential of benzoyl peroxide is as yet not fully answered, but at the present time it seems likely that this compound is safe to use.

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Etretinate and the risk for teratogenicity: drug monitoring in a pregnant woman for 9 months after stopping treatment

SIR, Teratogenicity is a possible side-effect of oral retinoid therapy and the risk is particularly great with etretinate, because of its storage in fat and its continued release after therapy has ceased.[1,2] Although the lowest dose for a teratogenic effect is not known, a suspected case of etretinate-induced foetal malformation was observed in a case where conception occurred almost a year after the last dose of the drug.[3] We report a case of a woman who became pregnant 7·5 months after stopping etretinate, and we determined the drug concentration in maternal blood and subcutaneous tissue as well as in the amniotic fluid.

In December 1985, a 28-year-old woman was prescribed etretinate for extensive discoid lupus erythematosus that was resistant to conventional therapy.[4] The patient was otherwise well with no signs of systemic involvement. Etretinate was given at a daily dose of 50 mg and treatment was discontinued in January 1986 because of mucocutaneous side-effects and an inadequate response to therapy. The patient was told by her dermatologist to remain on an oral contraceptive for at least 1 year following therapy, but she stopped in July 1986 because of irregular menstrual bleeding. Two months later she became pregnant, 32 weeks following treatment with etretinate. The patient was referred to us for evaluation of the risk for retinoid-induced foetal malformation.

In October 1986, the plasma and tissue levels of etretinate including its metabolites, acitretin and *cis*-acitretin, were determined using HPLC as previously described.[1] A concentration of 10 ng/ml was found in the plasma. A subcutaneous fat biopsy indicated a concentration of 1·21 μg/g, which is well within the usual range seen 9 months after treatment.[1] It was not possible to perform an amniocentesis until January 1987, when the foetus was 16 weeks old. Clear amniotic fluid (10 ml) was withdrawn for HPLC analysis. The etretinate and acitretin concentrations were below the detection limit of the assay (less than 0·4 ng/ml). The plasma concentration of the drug had now fallen to 3·6 ng/ml. A full gynaecological examination and analysis of the chromosomes of the amniotic cells revealed no abnormal findings. The patient wished to continue her pregnancy and 6 months later she delivered a healthy male child with no congenital malformations. A check-up of the child 1 year later revealed no abnormal findings.

The data on file from the manufacturers of etretinate (Hoffmann-La Roche Ltd, Basle, Switzerland) have so far indicated suspected teratogenicity in eight out of 95 foetuses reportedly exposed to the drug in the period 1–24 months following treatment. The incidence rate of major congenital malformation in the general population is in the order of 2–6%. The plasma and fat concentrations of etretinate below which the risk for teratogenicity is negligible are not known, and the current recommendation is for the patient to avoid pregnancy for at least 2 years following treatment with the drug.

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British Journal of Dermatology (1990) **123**, 132–133.

Book Reviews

Irritant Contact Dermatitis. Edited by E.M. Jackson and R. Goldner (1990). Marcel Dekker, New York: Marcel Dekker, Inc. Pp. 240. ISBN 0-8247-8288-7. Price: $119.50.

This book is part of a series which has recently been introduced to review particular aspects of experimental and clinical dermatology. The editors, a toxicologist and a dermatologist, have contributions from an international group of experienced dermatology practitioners, researchers, and occupational health physicians.

The book is divided into three sections: Inflammation, Exposures to Irritants and Tests for Irritants. Each of these sections is clearly subdivided into identifiable, well annotated sections. Each is supplied with a full and comprehensive list of references. The text itself is concise and designed to give an outline of each aspect of the irritant response. In the second section some indications as to sources and chemical nature of various irritants are covered, both in the domestic and occupational environment. Physical irritants are included in this section. The third section briefly describes the methods available for testing human and animal skin for irritancy, and brief reference here is made to standard patch testing, and of course allergic contact dermatitis as well.

It is an easily read and understood text and a sound introduction and summary of the vast field of irritant dermatitis. The figures are simple and straight forward and the text is complemented by tables to simplify explanatory passages. The chapter on photo contact and plant exposure has black and white photographs of some commonly found irritant plants.

Any dermatology library would benefit from inclusion of this book, and it is of a sufficiently general standard to be of help to all clinical and also research orientated dermatologists. One or two printer's errors have not been identified but the meaning of the text is not impaired in any way because of these. This book must be regarded as essential for those with a particular interest in contact dermatitis.

ANGELA FORSYTH

Acyclovir Therapy for Herpesvirus Infection. Edited by D.A. Baker (1990). New York: Marcel Dekker, Inc. Pp. 344 pages; 8 colour and 8 B+W photographs. ISBN 0-8247-8091-4. Price in U.S. $119.50.

Acyclovir is one of the best understood and most specific drugs in dermatology. Not only is it selectively activated in herpes-virus infected cells, but it then has a selective effect on replication of viral DNA within those cells. The excitement of this pharmacological concept is rather drowned in the sea of therapeutic details which are the main topic of this book.

Two introductory chapters cover the science and clinical pharmacology of acyclovir. The remainder deal mainly with herpes simplex infections, in chapters arranged largely by body site; the final chapters cover herpes varicella-zoster, and herpes infections in immunosuppressed subjects or patients with HIV infection. Some authors of the clinically orientated chapters have essentially adhered to the topic of acyclovir therapy suggested by the title of the book, whilst others have written more extensively about other aspects of herpes virus infection. This is often interesting but has led to a considerable degree of repetition of general information about herpes viruses, laboratory diagnosis, and antiviral therapies other than acyclovir. Most chapters also reiterate the mode of action, routes of administration, dosage and possible side-effects of acyclovir which could have been covered as a single chapter. A more detailed discussion of acyclovir resistance might have been appropriate given the title of the book, but the topics covered are otherwise fairly comprehensive and contain recent references.

The book contains a liberal sprinkling of spelling mistakes, including one chapter where 'vessicles' and vesicles are used as interchangeable terms. Numerous pages of the review copy had blurred print, and there are some incorrect cross-references and incorrect entries in the rather scanty index. Some editorial aberrations were amusing — I liked '. . . in the case of Whitlow's . . .' — but some sentences are incomprehensible. I expected better quality in a book at this price.

Dermatologists are likely to be rather disappointed by the content of the book. The sections on erythema multiforme and eczema herpeticum are each rather shorter than this review, and there is minimal discussion of the role of acyclovir in patients with recurrent herpes simplex-associated erythema multiforme. The chapter on suppressive therapy with acyclovir deals mainly with genital herpes, and that on acyclovir for herpes zoster is somewhat less helpful than a recent issue of *Current Problems in Dermatology* by the same author (J.C.Huff). The chapters dealing with genital herpes are more detailed and the book may be more useful to venereologists; I cannot recommend it for personal purchase by dermatologists.

N.H.Cox

The Look You Like: Medical Answers to 400 Questions on Skin and Hair Care. LINDA A.SCHOEN AND P.LAZAR (1990). New York, Basle: Marcel Dekker Inc. Pp. 325. ISBN 0-8247-8146-5.

The 1980s saw a further increase in fitness and exercise mania and a striving to physical perfection by many of the British population. Like many trends this had spread over from America and seems to have caught on. Not surprisingly the trend was accompanied by a host of books on how to achieve the body beautiful including the perfect skin. The skin books were in the main purely aimed at improving the texture and appearance of the skin. Over the same time completely separate publications aimed at those with the commoner skin diseases like acne and psoriasis have appeared. The authors of '*The Look You Like*' have tried to meet both aims in one publication. Their book which is sponsored by the American Academy of Dermatology attempts to span the divide between cosmetic and therapeutic dermatology. Its target audience, according to the dustcover is wide; it includes dermatologists, other physicians, beauty therapists and the general public.

The book consists of a series of questions and answers on topics like hair and nail care, anti-ageing strategies and general skincare advice interspersed among those on bona fide skin diseases like acne, psoriasis and the alopecias. These latter conditions are well explained and would make useful reading for patients to augment information obtained during a dermatological consultation. The aetiology of the diseases is given together with an up-to-date account of possible treatments and their side-effects. Various myths held by the general public like the infective nature of psoriasis and the effect of diet on acne are exploded. There is a good section on skin cancers preceded by a chapter on sensible sun exposure and the possible side-effects of both UVB and UVA. People are encouraged to examine their skin regularly for pigmented lesions and to use the already well publicized ABCD checklist.

Interspersed among this information are chapters on the more cosmetic aspects of dermatology. The topics include general skin care, hair 'treatments' like dyeing and perming and care of the hands and feet. A section is devoted to sports related skin problems listing such terrors as swimmers ear, joggers nipples and exercise machine acne as a final deterrent to the sloths among us. While the questions in these sections are unlikely to be the reason for referral to a busy NHS dermatology clinic I suspect that they are often directed at general practitioners as a 'while I'm here, doctor' request.

In general I thought the information in the book was clear and sensible. I would doubt that it would achieve the wide audience in Britain indicated by the American authors. It would best be aimed at hair and beauty specialists and informed members of the public. In addition many of our patients could benefit from being directed to the chapters on the common skin diseases. If, however, British dermatology follows its American counterparts' path to increasing consultations on cosmetic rather than therapeutic dermatology perhaps we should reserve our copy of this book now.

VALERIE R.DOHERTY

British Journal of Dermatology (1990) **123**, 134–135.

News and Notices

Netherlands Association for Dermatology and Venereology
Biennial Prize

The Netherlands Association for Dermatology and Venereology awarded their biennial prize, the Professor D.van Haren-Noman prize, value 15,000 guilders, to Dr Peter van de Kerkhof of the Department of Dermatology in Nijmegen. This was for his work on the role of the polymorphonuclear leucocyte in psoriasis.

Dermatology '90: Therapeutic Update
21–23 September 1990

A meeting will be held to look at current and future drug treatment of skin diseases. Dr John Dunne of the World Health Organization in Geneva will present the keynote address and other speakers will include John Voorhees and Keith Johnson. This meeting is to be held at New World Harbourside, Vancouver BC. For further information and registration call (604) 669-7175 or fax (604) 669-7083 or write to Dermatology '90, 204-402 W. Pender Street, Vancouver, BC V6B 1T6, Canada.

The Skin Disease Research Fund

The Skin Disease Research Fund invites applications for grants, and the deadline for submissions is 3 September 1990. The Fund's own forms can be obtained from the Honorary Appeal Secretary Mr D.K.G.Gowers, 12 Knighton Close, South Croydon, Surrey CR2 6DP (Tel: 081-668 4300). Further information regarding the grants policies of the Fund may be found in the February 1988 issue of the *British Journal of Dermatology* page 313.

19th World Congress of Dermatology in 1997

The International League of Dermatological Societies (ILDS) wishes to announce that the 19th World Congress of Dermatology is scheduled to take place in 1997.

Applications are sought from Member Societies of the ILDS wishing to host the next World Congress in their countries. The final decision will be made by the assembly of delegates to the International League of Dermatological Societies in New York at the 1992 World Congress.

Applications should be prepared in as much detail as possible including information concerning the proposed organizing committee, site, and conference facilities. The Secretary General of the forthcoming New York Congress, Dr Stephen Katz, National Cancer Institute, Dermatology Branch, Building 10, Room 12N238, Bethesda MD 20892, U.S.A. (phone (301) 496-2481), is willing to provide advice on all necessary requirements needed for organizing the Congress. The applications should be sent to the Secretary General of the League, Dr Stuart Maddin, Division of Dermatology, University of British Columbia, 855 West 10th Avenue, Vancouver, BC Canada V5Z 1L7, phone (604) 874-6112, by 31 December 1991.

European Hair Research Society

A European Hair Research Society was founded in November 1989 and the objective is the promotion of scientific research in hair biology in health and disease and related subjects. Further information can be obtained from Dr D.Van Neste, Secretariat EHRS, Skinterface sprl, 9 rue du Sondart, B 7500 Tournai, Belgium.

The next meeting of the Society will be held in Bologna, 13–14 April 1991 and further information can be obtained from Professor A.Tosti, Istituto di Clinica Dermatologia, University of Bologna, Via Massarenti 1, 140138 Bologna, Italy.

Environmental Threat to the Skin

International Symposium, University of Wales College of Medicine, 15–17 April 1991

An International meeting on the Environmental Threat to the Skin will be held at the University of Wales College of Medicine, Cardiff on 15–17 April 1991. The meeting is being co-organized by Professor Ronald Marks of the University of Wales College of Medicine and Professor Gerd Plewig of the University of Dusseldorf. The meeting will consist of invited papers, free communications, a poster session and exhibits on the effects of environmental hazards on the skin, their prevention and treatment. For further details and abstract forms, please contact: Professor R.Marks, Professor of Dermatology, University of Wales College of Medicine, Heath Park, Cardiff CF4 4XN, U.K.

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NOTICE TO CONTRIBUTORS

The *British Journal of Dermatology* publishes original articles on all aspects of the normal biology, and of the pathology of the skin. Originally the Journal, founded in 1888, was devoted almost exclusively to the interests of the dermatologist in clinical practice. However, the rapid development, during the past twenty years, of research on the physiology and experimental pathology of the skin has been reflected in the contents of the Journal, which now provides a vehicle for the publication of both experimental and clinical research and serves equally the laboratory worker and the clinician. To bridge the gap between laboratory and clinic the Journal publishes signed editorials reviewing, primarily for the benefit of the clinician, advances in laboratory research, and also reviews of recent advances in such aspects of dermatology as contact dermatitis, therapeutics and drug reactions. Other regular features include book reviews, correspondence and society proceedings. The Journal is the official organ of the British Association of Dermatologists and also of the Netherlands Association for Dermatology and Venereology, but it attracts contributions from all countries in which sound research is carried out, and its circulation is equally international.

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gram(s)	g	minute(s)	min	molar	mol/l
kilogram(s)	kg	centimetre(s)	cm	millilitre(s)	ml
milligram(s) (10^{-3}g)	mg	second(s)	s	gravitational acceleration	***g***
microgram(s) (10^{-6}g)	μg	cubic millimetre(s)	mm^3	micrometre(s)	μm
nanogram(s) (10^{-9}g)	ng	millimetre(s)	mm	per cent	%
picogram(s) (10^{-12}g)	pg	millicurie(s)	mCi	isotopic mass number placed as	^{131}I
hours(s)	h	milliequivalent	mmol		

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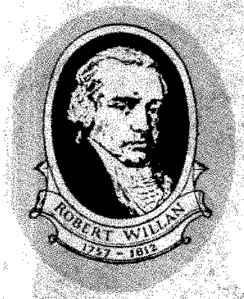

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107 Necrolytic migratory erythema: apparent response to oral omega-3 (marine) essential fatty acids; T.J.DELANEY AND J.S.UFF.

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112 Pemphigus vegetans masquerading as multiple squamous cell carcinomata; VANESSA VENNING, S.M.BURGE, K.DALZIEL AND P.R.MILLARD.

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117 Human orf and milkers' nodules: a clinico-pathological study. R.W.GROVES, E.WILSON JONES AND D.M.MACDONALD.

118 Cutaneous necrosis in chronic renal failure; N.P.BURROWS AND R.RUSSELL JONES.

119 Mast cells after capsaicin application in normal human skin; C.B.BUNKER, R.CERIO, H.A.BULL, J.EVANS, J.C.FOREMAN AND P.M.MCDOWD.

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120 Melanocyte atypia and melanoma; M.E.FALLOWFIELD AND M.G.COOK.

121 The histogenesis of lamellar fibrosis: a new concept; R.CERIO AND E.WILSON JONES.

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Foreword

This supplement contains details of the programme and summaries of the papers, posters and clinical cases to be presented at the 70th Annual Meeting of the British Association of Dermatologists, to be held in Warwick from 18 to 21 July 1990. Details of the papers and cases to be presented at the meeting of the British Society for Dermatopathology on 19 July 1990 are also included.

Once again, we are most grateful to Glaxo Laboratories for their most generous financial assistance towards the cost of printing this supplement.

S.S.BLEEHEN

DR R.R.M.HARMAN
President of the British Association of Dermatologists
1989–1990

Bristol Royal Infirmary

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British Association of Dermatologists Seventieth Annual Meeting, 1990, Warwick

PRESIDENT DR R.R.M.HARMAN

PROGRAMME OF MEETING

Wednesday 18 July 1990

08.00	Registration opens in Arts Centre, University of Warwick
08.00	Pharmaceutical Exhibition opens in Arts Centre Gallery
08.20	Introductory remarks
08.30–17.00	Scientific Session in Arts Centre Theatre
14.00–17.00	British Dermatological Surgery Group Meeting
14.00–17.00	British Society for Paediatric Dermatology Meeting
18.30–19.30	President's Reception, Panorama Room, University of Warwick

Thursday 19 July 1990

08.00	Pharmaceutical Exhibition opens in Arts Centre Gallery
08.15	Registration desk opens in Arts Centre
08.30–11.00	Annual General Meeting (members only)
11.30–17.00	Scientific Session in Arts Centre Theatre
14.00–17.00	British Contact Dermatitis Group Meeting
14.00–17.00	British Society for Dermatopathology Meeting
Afternoon	Golf competition at Coventry Golf Club

Friday 20 July 1990

08.00	Pharmaceutical Exhibition opens in Arts Centre Gallery
08.15	Registration desk opens in Arts Centre
08.30–13.00	Scientific Session in Arts Centre Theatre
14.00–17.00	Scientific Session in the Conference Room
14.00–17.00	Photodermatology Group Meeting
Afternoon	Sailing Competition at Draycote Water
19.00 for 19.30	Annual Dinner, Chesford Grange Hotel, Kenilworth

Saturday 21 July 1990

08.30–13.00	Clinical Pathological Conference Session in Arts Centre Theatre

Wednesday 18 July 1990

08.00	Registration desk opens in Arts Centre, University of Warwick	
08.00	Pharmaceutical exhibition opens	
08.20	Introductory remarks	
08.30	C.L.Wilson, S.M.Burge, D.Dean, R.P.R.Dawber and F.Wojnarowska	A clinicopathological study of scarring alopecia in chronic cutaneous lupus erythematosus
08.45	G.M.Murphy and J.L.M. Hawk	The incidence of positive antinuclear antibodies and lupus erythematosus in patients presenting with apparent polymorphic light eruption
09.00	J.Jackson and L.Barnes	Anti-neutrophil cytoplasmic antibodies in dermatitis herpetiformis
09.15	V.Venning, P.H.Whitehead, I.M.Leigh, J.Allen and F.Wojnarowska	Clinical variants of bullous pemphigoid are not determined by the specificity of the target antigen defined by immunoblotting
09.30	M.R.Judge, B.D.Lake, V.V.Smith, G.T.Besley and J.I.Harper	Sjögren-Larsson syndrome: a disorder of fatty alcohol metabolism
09.45	R.Cerio, N.P.Smith and E.Wilson Jones	Xanthoma disseminatum and histiocytosis X: rare dermatoses easily confused
10.00	S.M.Vijayasingam, P.J.Dykes and R.Marks	Phenytoin has little effect on models of wound healing *in vitro*
10.15	D.Black and R.Marks	Age influence on topical corticosteroid effects on skin
10.30	**Coffee**	
11.00	**Guest Lecture** Professor P.J.Barnes	Mechanisms of allergic inflammation
11.45	H.M.Goodyear, P.W.Laidler, E.H.Price, A.P.Kenny and J.I.Harper	A clinico-microbiological study of toxic erythemas in children
12.00	J.Berth-Jones and P.E.Hutchinson	Response rates to current treatment of common warts and evaluation of continued cryotherapy and inosine pranobex for treatment failures

12.15	P.Duhra, D.Catty, D.Jassim and S.D'Arcy	A serological test for rapid diagnosis of cutaneous tuberculosis
12.30	D.W.S.Harris, E.C.Benton and J.A.A.Hunter	Dermatological audit: facts or friction
12.45	A.J.G.McDonagh, D.J.Gawkrodger and A.E.Walker	Chloracne: report of an outbreak with new clinical observations
13.00	**Lunch**	
14.00	R.J.Motley and A.Y.Finlay	Practical use of a disability index in the routine management of acne
14.15	D.M.Tillman, S.I.White and T.C.Aitchison	Isotretinoin, creatine kinase and exercise
14.30	N.Nazmi, L.R.Lever and R.Marks	Effect of isotretinoin on epidermal proliferation
14.45	J.Garioch, P.Todd, M.Rademaker and A.Forsyth	Allergic contact dermatitis reactions to latex examination gloves
15.00	J.Ashworth, I.R.White, R.J.G.Rycroft and E.Cronin	Contact sensitivity to topical corticosteroids
15.15	P.H.Cartwright, S.M.Wilkinson and J.S.C.English	Tixocortal pivalate is a marker for hydrocortisone sensitivity
15.30	**Tea**	
16.00	J.Garioch, P.Todd, P.J.Lamey, M.Lewis, A.Forsyth and M.Rademaker	The significance of a positive patch test to mercury in oral disease
16.15	P.Todd, J.Garioch, P.J.Lamey, M.Lewis, A.Forsyth and M.Rademaker	Patch testing in lichenoid reactions of the mouth and oral lichen planus
16.30	V.R.Doherty, A.Forsyth and R.M.MacKie	Pruritus vulvae: a manifestation of contact hypersensitivity?
16.45	A.J.Carmichael, I.S.Foulds and D.Bransbury	Loss of lanolin patch test positivity
18.00	Closure of Pharmaceutical Exhibition	

Thursday 19 July 1990

08.00	Pharmaceutical Exhibition opens
08.15	Registration desk opens in Arts Centre
08.30	Annual General Meeting (members only)
11.00	**Coffee**

11.30	**Guest Lecture**	
	Professor J.O.Martey	The development of a School of Medicine in West Africa
12.15	M.E.Murdoch, R.J.Hay, N.Ramnarain, S.Lucas, B. Jones and A. Abiose	A clinical classification and grading system of the cutaneous changes in onchocerciasis and histopathological findings
12.30	R.H.Bull and R.C.D. Staughton	Increased risk of cutaneous reactions to Fansidar in HIV disease
12.45	S.Whittaker, M.Rustin, G.Levene, D.Thompson and N.Smith	HTLV-1 associated cutaneous disease
13.00	**Lunch**	
14.00	W.A.D.Griffiths	Nail-blanching: a new cutaneous sign indicating mechanical and functional impairment of the finger pulp
14.15	J.E.White, P.Perkins and E.G.V.Evans	Successful treatment of chronic tinea pedis and tinea manuum with Lamisil® (terbinafine)
14.30	A.Y.Finlay, L.Lever, R.Thomas and P.J.Dykes	Nail matrix kinetics of systemic terbinafine in onychomycosis
14.45	C.C.Harland, M.J.D.Goodfield and E.G.V.Evans	A follow-up study of terbinafine (SF-327, Lamisil®) in the treatment of onchomycosis
15.00	D.A Fenton, R.Bull, J.Gane and P.S.Mortimer	Abnormal lymphatic function assessed by quantitative lymphoscintigraphy in yellow nail syndrome
15.15	R.H.Bull, P.S.Mortimer, J.Gane and A.G.A.Joseph	Abnormal lymph drainage in patients with chronic venous leg ulcers
15.30	**Tea**	
16.00	D.V.Stevanović, A.Krunić and Z.Djaković	Pathogenesis in bamboo hair
16.15	D.A.Fenton, D.J.Tobin, M.M.Black and M.D.Kendal	Incomplete melanogenesis with melanocyte abnormalities in acute alopecia areata

16.30	S.C.Mayou, C.Ridler and J.D.Kirby	Haemostatic abnormalities in Sneddon's syndrome
16.45	C.C.Harland, R.J.Madeley and L.G.Millard	Patient information leaflets in the dermatology out-patient department
18.00	Closure of Pharmaceutical Exhibition	

Friday 20 July 1990

08.00	Pharmaceutical exhibition opens in Arts Centre Gallery	
08.15	Registration desk opens in Arts Centre	
08.30	M.T.Glover, B.D.Lake and D.J.Atherton	Infantile systemic hyalinosis: a newly recognized disorder of collagen?
08.45	R.A.J.Eady, D.B.Gunner, B.D.Lake, A.H.M.Heagerty, O.M.V.Schofield, J.Allen, P.MacKinnon, K.H.Nicolaides and C.H.Rodeck	Prenatal diagnosis of genetic skin disease using foetal skin biopsy: 10 years' experience
09.00	A.J.G.McDonagh, A.L.Wright, D.W.K.Cotton and S.S.Bleehen	Malignant melanoma in Sheffield 1971–88
09.15	R.A.C.Graham-Brown, J.E.Osborne, S.P.London and A.Fletcher	Public education for melanoma in Leicestershire: an update
09.30	C.Edwards, R.Heggie and R.Marks	*In vivo* measurement of melanin pigmentation
09.45	A.W.P. du Vivier, H.C.Williams, J.V.Brett and E.M.Higgins	How do malignant melanomas present and does this correlate with the 7-point check list?
10.00	R.M.MacKie, R.Bufalino and C.Sutherland	The effect of pregnancy on melanoma prognosis
10.15	S.D.Shalom, I.Harvey, R.Marks and S.J.Frankel	The prevalence of solar keratoses in a South Wales population
10.30	**Coffee**	
11.00	**Guest Lecture**	
	Dr H.Grossman	Priorities in dermatology in developing countries. A challenge
11.45	C.M.Lawrence	Only cartilage needs to be removed in the surgical treatment of chondrodermatitis nodularis

12.00	P.Duhra, C.Hodgson, A.Ilchyshyn and D.I.Porter	Bath PUVA for psoriasis: the West Midlands experience
12.15	R.A.Sheehan-Dare, M.J.D.Goodfield and N.R.Rowell	The influence of indomethacin on disease activity in patients with psoriasis during treatment with the Ingram regime
12.30	A.V.Powles, B.S.Baker, H.Valdimarsson and L.Fry	Four years' experience of cyclosporin for psoriasis
12.45	A.M.Layton, R.A.Sheehan-Dare and W.J.Cunliffe	A double-blind placebo-controlled trial of topical PUVA in persistent palmoplantar pustulosis
13.00	**Lunch**	
14.00	C.Guzzo and G.Lazarus	Urticaria pigmentosa: systemic evaluation and successful treatment with topical steroids
14.15	C.E.H.Grattan, D.M.Francis and M.W.Greaves	A histamine releasing factor in serum of chronic urticaria with anti-IgE autoantibody-like properties
14.30	R.C.H.Yu, D.W.Pryce, A.W.MacFarlane and T.W.Stewart	A histopathological study of 643 cutaneous horns
14.45	L.Shall, L.G.Millard, A.Stevens, R.B.Tattersall and I.Peacock	Necrobiosis lipoidica: 'the footprint not the footstep'
15.00	M.P.Sheehan, D.J.Atherton, V.Broadbent and J.Pritchard	Topical mechlorethamine: an effective treatment for skin involvement in Langerhans cell histiocytosis
15.15	A.Anstey, M.Winter and F.M.Pope	Platelet and coagulation studies in Ehlers Danlos syndrome
15.30	**Tea**	
16.00	S.C.Mayou, J.D.Kirby and I.B.Kovacs	Infusion of iloprost in Raynaud's phenomenon: lack of antiplatelet effect but risk involved
16.15	J.M.Sowden, J.H.Ransom, R.J.Powell and B.R.Allen	Activated peripheral blood T lymphocytes in atopic dermatitis
16.30	M.Judge and J.Harper	The ichthyoses: a re-appraisal based on a study of 100 patients

16.45	J.S.Ross, P.G.Norris, J.L.M.Hawk and R.D.R.Camp	Long-term cyclosporin A in eczematous disorders
16.15	Closure of Pharmaceutical Exhibition	

Poster presentations

N.Burrows and R.Russell Jones	Pretibial epidermolysis bullosa: a report of three families
R.Cerio, T.S.Sonnex, N.P.Smith and E.Wilson Jones	Desmoplastic melanoma: a rare form of malignant melanoma that is usually mis-diagnosed and mis-managed
G.W.Cherry, S.Sockalingen, C.S.Clay, J.Cameron, C.A.Cherry, J.C.Barbenel, T.J.Ryan, D.Queen and M.Frank	Evaluation of a unique new compression bandage in the treatment of venous ulcers
D.de Berker, S.M.Burge, M.Dissanayake and F.Wojnarowska	The sequelae of chronic cutaneous lupus erythematosus
D.J.Eedy, C.F.Johnston, C.Shaw and K.D.Buchanan	Peptidergic neurotransmitter innervation of the eccrine sweat gland and their presence in sweat secretions
M.J.Finnen and C.R.Lovell	Inhibition of human epidermal phospholipase A2 by polyunsaturated fatty acids
R.Herriot, A.D.Ormerod and H.F.Sewell	An immunophenotypic and flow cytometric investigation of adult mastocytosis
F.Humphreys	H1 antagonism reveals the non-histamine component of antigen weals: a model for further study?
S.K.Jones and H.Moseley	Clear UV lenses: cosmetically acceptable spectacles for PUVA patients
M.Keefe, J.E.White and P.Perkins	Nodular melanomas in the over-50 age group: the next target for health education
S.E.Kelly, S.Fleming and M.M.Black	The comparative immunology of the specific dermatoses of pregnancy
D.Kemmett and R.St.C.Barnetson	Topical nedocromil sodium: a double-blind placebo-controlled study in atopic dermatitis
C.R.Lovell	Important plant allergens in the United Kingdom
J.McFadden, J.N.Leonard, A.Powles, H.Valdimarsson and L.Fry	Homology between glutenin and human connective tissue proteins
S.M.Neill, J.P.Ellis, A.C.Pembroke and R.C.D.Staughton	Plantar fibromatosis
S.M.Neill, R.C.D.Staughton, E.B.Lane and I.M.Leigh	The cytokeratin profile of normal vulval epithelium and vulval lichen sclerosus
F.Oliver, E.U.Amon, A.Breathnach, A.Kobza Black, D.M.Francis, P.Sarathchandra and M.W.Greaves	Contact urticaria due to the common stinging nettle (*Urtica dioica*): histological, ultrastructural and pharmacological studies
N.J.Reynolds, C.N.A.Matthews and J.L.Burton	Strimmer dermatitis

W.Robles Martinez, B.Bhogal, C.A.Morrell, M.K.Moore and R.J.Hay	The use of fluorescent lectin stains to identify fungi in clinical material from skin
J.S.Ross, K.B.Bacon, N.J.Fincham, A.J.H.Gearing and R.D.R.Camp	Mediators of leucocyte infiltration in human skin: a review of recent findings
J.A.Salisbury, M.Glover, I.M.Leigh and H.Baker	The PUVA-associated squamous cell carcinoma: a distinct clinico-pathological entity
O.M.V.Schofield, J.-P.Cassella, H.A.Navsaria, I.M.Leigh, B.J.Mayou and R.A.J.Eady	Cultured keratinocyte allografts in dystrophic epidermolysis bullosa: preliminary observations
S.Sibenge and D.J.Gawkrodger	Rosacea: a survey of presentation patterns and response to therapy
J.P.Vestey, D.L.Yirrell and R.D.Aldridge	A case of human catpox/cowpox infection
Guest Poster	
G.M.Murphy, R.Fedler and P.D.Samman	St. John's Hospital for Diseases of the Skin: past and future

The following American Academy of Dermatology Award-Winning Posters will also be on display

Gold Award	
R.K.Curley and R.A.Marsden	Iododerma: a potentially fatal facial eruption
Silver Award	
K.Dalziel and R.P.R.Dawber	The effects of drugs on hair growth
Bronze Awards	
C.C.Harland, L.Shall and B.R.Allen	Historical overview: the thalidomide saga in dermatology
A.Milligan, P.E.Hutchinson and A.Fletcher	The Hardy microtome: a 'new' and reliable method of hair cross-section measurement
G.M.Murphy and J.L.M.Hawk	Sunbed-induced pseudoporphyria
E.Willsteed, B.Bhogal and M.M.Black	Artefacts in immunofluorescence microscopy: a potential cause of diagnostic confusion

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SUMMARIES OF PAPERS

A clinicopathological study of scarring alopecia in chronic cutaneous lupus erythematosus

C.L.WILSON, S.M.BURGE, D.DEAN, R.P.R.DAWBER AND F.WOJNAROWSKA

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Discoid lupus erythematosus (DLE) is a destructive form of chronic cutaneous lupus erythematosus (CCLE). Scarring involves dermal connective tissue and skin appendages, particularly hair follicles, but the pathogenesis of DLE is poorly understood. Factors which play a part in determining the likelihood of scarring may include the age and sex of the patient, the chronicity of the lesion and previous treatments. Sun damaged skin with abnormal elastic tissue may also be more likely to scar. The hair follicle is a useful model for the investigation of scarring pathology and we have examined the clinical and histological features in a group of patients with DLE and scarring alopecia.

Scalp involvement was present in 28% (10 males and 15 females) of 85 patients with DLE: 14 patients had localized DLE and 10 had generalized DLE. Scalp involvement was an early feature in 56% of patients; 63% gave a history of photosensitivity but there was no unifying pattern to the distribution of scalp involvement and no obvious correlation with sun exposure. Ten patients gave informed consent to further investigation. Biopsies were taken from clinically normal scalp, clinically active LE, and from the edge of an area of scarring alopecia.

Biopsies were assessed blind. Alopecia was not associated with an increase in the telogen count. Solar elastosis was more prominent in active and scarred areas but sebaceous glands were reduced in size or absent. Epidermal involvement was not a prominent feature. There was a patchy perifollicular lymphocytic infiltrate around mid-dermal vessels and the middle portion of the follicles. The upper and lower portions of the follicle were relatively spared.

We conclude that the pathognomonic histological features seen in epidermal LE[1] may be absent in scalp LE. The trigger for involvement of the middle portion of the hair follicle is uncertain, but in normal scalp there is often a sparse infiltrate in this zone and there is a change in the expression of keratins from simple to stratified squamous in this portion of the follicle.

REFERENCE

1 Bangert JL, Freeman RG, Sontheimer RD, Gilliam JN. Subacute cutaneous lupus erythematosus and discoid lupus erythematosus. *Arch Dermatol* 1984; **120**: 332–37.

The incidence of positive antinuclear antibodies and lupus erythematosus in patients presenting with apparent polymorphic light eruption

G.M.MURPHY AND J.L.M.HAWK

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Polymorphic light eruption (PLE) is an idiopathic photodermatosis characterized by a variable usually papular erythematous rash occurring hours after ultraviolet radiation exposure. One of the important differential diagnoses is lupus erythematosus (LE). We audited our practice of screening patients with a history of PLE for LE, by examining 1988–89 out-patient records: 142 patients thought to have PLE were screened for antinuclear (ANA), Ro and La antibodies. Sixty-six had negative findings (mean age 35, range 8–63 years). Sixty-two had ANA of various patterns, ranging from trace to titres of 1/80, thought not to be significant (mean age 35, range 11–65 years). Fourteen (mean age 42, range 16–65 years) had significant findings: 10 with ANA ranging in titre from 1/160 to 1/1280, six with Ro antibodies, four of whom had negative or low titre ANA. Two ANA and Ro positive patients had La antibodies. Three ANA positive patients fulfilled the American Rheumatism Association criteria for diagnosis of systemic LE. One patient with ANA 1/20 and strongly positive Ro had subacute cutaneous LE. One patient with negative ANA, Ro and La antibodies developed discoid LE. In 10 patients it was not clear whether the PLE-like rash represented cutaneous LE, or coincidental PLE, thus follow-up of these patients is essential. Cutaneous LE may present with a history identical to PLE. In this series, 10% of patients initially thought to have PLE had either significant ANA or Ro and La antibodies. All patients with apparent PLE should therefore be screened for LE.

Anti-neutrophil cytoplasmic antibodies in dermatitis herpetiformis

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Anti-neutrophil cytoplasmic antibodies (ANCA) have been reported in association with Wegener's granulomatosis, polyarteritis nodosa and in patients with systemic lupus erythematosus. Recently ANCA have also been described in patients with acute febrile neutrophilic dermatosis (Sweet's syndrome). In this study the incidence of ANCA in patients with dermatitis herpetiformis was examined.

An indirect immunofluorescence technique using alcohol-fixed smears of human neutrophils was established. Coded sera from 23 patients with dermatitis herpetiformis, four patients with Wegener's granulomatosis, 21 patients with Crohn's disease, 26 patients with ulcerative colitis and 31 normal controls were examined for the presence of ANCA. Positive results were found in 17% (4) of patients with dermatitis herpetiformis, 100% of patients with Wegener's granulomatosis, 43% of Crohn's disease, 27% of ulcerative colitis and 0% of normal controls. The staining pattern observed in the patients with dermatitis herpetiformis and inflammatory bowel disease was a diffuse cytoplasmic pattern as opposed to the patterns which occur in patients with vasculitis. In one patient with dermatitis herpetiformis and ANCA, disappearance of the antibody from the circulation was associated with clinical remission. These findings suggest that ANCA may be produced in a variety of neutrophilic dermatological disorders. Furthermore, particular types of ANCA are associated with distinct disease states.

Clinical variants of bullous pemphigoid are not determined by the specificity of the target antigen defined by immunoblotting

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Immunoblotting and immunoprecipitation studies suggest the major bullous pemphigoid (BP) antigen is a 220-240 kDa polypeptide,[1] though some studies suggest heterogeneity of the BP antigen(s), with sera recognizing bands of 180–200 kDa or lower molecular weight.[2] As bullous pemphigoid can show considerable variation in its clinical presentation and course, we investigated whether heterogeneity of the antigen accounted for this variation.

Sera from 39 patients with BP were studied by immunoblotting using extracts of NaCl-separated epidermis, and the results were compared with a reference BP serum producing a band at 220 kDa (positive control) and with normal human serum. Ten sera produced a single band at MW 220 kDa, and two sera produced a major band at 220 kDa, with multiple indistinct bands in the 180–200 kDa range. Two sera produced a single band at MW 180 kDa, and 25 sera identified no antigens in the immunoblots, despite detectable antibody on indirect immunofluorescence in 14/25. Of the 12 sera identifying a 220 kDa antigen, nine were from patients with morphologically typical lesions but three were from patients with clinically unusual BP (two localized, one with prurigo-like skin lesions and oral erosions). Mucosal lesions occurred in 6/12, milia in 2/12, positive indirect immunofluorescence (IIMF) in 12/12. Both the 180 kDa sera were from patients with morphologically typical skin lesions and mucosal involvement, and one also had milia. One was unusual in that she developed an unexplained oesophageal web 4 years after diagnosis of BP. IIMF was negative in one and positive (1/10) in the other. Of the 25 sera identifying no antigens on immunoblots, 20 patients had morphologically typical lesions, and four were clinically unusual (one localized, two annular erythema-like lesions, one with recalcitrant erosions of the hands). Mucosal lesions occurred in 14/25, milia in 8/25 and positive IIMF in 15/25.

We confirm that the major BP antigen is a 220 kDa polypeptide and that the 180 kDa polypeptide is a second BP antigen and sometimes the sole antigen identified in immunoblots. There was no correlation between the BP antigens detected and the clinical presentation and course of BP; factors other than antigen specificity must therefore determine the clinical expression of BP.

REFERENCES

1 Mueller S, Klaus-Kovtan V, Stanley JR. A 230-kD basic protein is the major bullous pemphigoid antigen. *J Invest Dermatol* 1989; **92:** 33–38.

2 Labib RS, Anhalt GJ, Patel HP, Mutasim DF, Diaz LA. Molecular heterogeneity of the bullous pemphigoid antigens as detected by immunoblotting. *J Immunol* 1986; **136:** 1231–35.

Sjögren-Larsson syndrome: a disorder of fatty alcohol metabolism

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We have studied seven patients with Sjögren-Larsson syndrome, an autosomal recessive disorder which comprises ichthyosis, mild to moderate mental retardation and a spastic diplegia or tetraplegia. Five of them had the characteristic maculopathy. We have demonstrated a reduction of the enzyme, fatty alcohol oxidoreductase, in skin fibroblasts and leucocytes in all of these patients, confirming the findings of Rizzo *et al.*[1]

In addition, we have developed a histochemical technique which demonstrates a reduction in alcohol dehydrogenase activity in the epidermis of the seven, and in jejunal mucosa of the three patients who had jejunal biopsies. Controls included 20 patients with a variety of other ichthyoses, disorders of keratinization such as porokeratosis and samples of normal skin, all of which showed normal alcohol dehydrogenase activity. This hopefully will prove to be a useful diagnostic test for Sjögren-Larsson syndrome.

REFERENCE

1 Rizzo WB, Dammann AL, Craft DA *et al.* Sjögren-Larsson syndrome: inherited defect in the fatty alcohol cycle. *J Pediat* 1989; **115**: 228–34.

Xanthoma disseminatum and histiocytosis X: rare dermatoses easily confused

R.CERIO, N.P.SMITH AND E.WILSON JONES

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Xanthoma disseminatum (XD) and subacute forms of histiocytosis X (HX) share several clinicopathological similarities. These include yellow/brown papulonodular lesions often affecting the flexures and the mouth and an association with diabetes insipidus. Histologically, both show upper dermal infiltration with 'histiocytoid' cells accompanied by inflammatory cells, including eosinophils. Both disorders are rare and differential diagnosis is often difficult.

We reviewed the clinicopathological features of six patients with XD (three female, three male; mean age 37 years) and 10 patients with HX (four female, six male; mean age 19 years). Both had prominent skin involvement. Skin biopsies were routinely paraffin processed. Sections were examined using various conventional tinctorial stains and immunolabelled for S100 protein and the recently developed monocyte/macrophages: Mac 387 (L1 antigen), Factor XIIIa (dermal dendrocytes) and KP1 (CD68).

There were striking similarities in distribution of the lesions, although diabetes insipidus was not a feature. Microscopically, Touton giant cells were prominent in XD and many large 'histiocytoid' cells in HX had indented nuclei. Exocytosis of the latter was encountered in HX and multinucleate cells were rare. Immunophenotypic studies showed differential labelling. In XD giant cells were identified with KP1 and several mononuclear 'histiocytoid' cells were Mac

387$^+$ and Factor XIIIa$^+$ but S100 protein negative. In contrast, the 'histiocytoid' cells of HX were S100 protein positive.

Despite the clinical and pathological similarities sometimes observed in XD and some forms of HX, our studies demonstrate that immunocytochemical labelling employing S100 protein and new monocyte/macrophage markers in paraffin sections may aid differential diagnosis. Moreover, such studies may further help us understand the histogenesis of this rare group of disorders.

Phenytoin has little effect on models of wound healing *in vitro*

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Many reports have appeared documenting beneficial effects to persistent wounds and ulcers of topically applied phenytoin. These reports, in conjunction with others, that patients taking the drug by mouth develop gingival hyperplasia, have suggested that it may have stimulatory effects on fibrous and epithelial tissues. To investigate the possibility further, we have performed the following studies.

Fibroblasts and keratinocytes derived from human foreskins were cultured in Dulbecco's modified Eagle's medium with 10% fetal calf serum (FCS) containing phenytoin (in concentrations of 10^{-4} to 10^{-9} M). Duplicate cultures were set up containing 1% FCS only. Controls were also set up containing alcohol solvent instead of phenytoin. The growth of the cells in these cultures was assessed by colony size estimations on an Optomax 2 image analyser. Effects on epidermal migration were assessed using the 'epiboly phenomenon' (2 mm^2 free-floating explants of normal whole skin) in similar conditions to those used for cell culture. The degree of epiboly from the explants was assessed by an image analysis method using the same Optomax device.

The results indicate that there was no appreciable effect on cell culture (e.g. fibroblasts in 10% FCS, after 2 days culture, showed average percentage growths of 89·9% for control vs. 83·5% for 10^{-5} M and 91·8% for 10^{-9} M concentration of phenytoin, while keratinocytes in 10% FCS after 5 days showed values of 33·7% for controls vs. 36·2% for 10^{-5} M and 23% at 10^{-9} M concentrations of phenytoin), and no consistent effects on epidermal migration after 48 hr (e.g. in 10% FCS, epiboly doubled at the 10^{-6} M concentration but fell almost back to control values at 10^{-7} M, only to rise again between 10^{-8} and 10^{-9} M levels of phenytoin). These results emphasize the need for a critical approach to therapeutic claims in the wound-healing area, especially when they are based on uncontrolled and anecdotal reports.

Age influence on topical corticosteroid effects on skin

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Human pharmacology studies of topical corticosteroids are not generally conducted with the possible effects of age in mind. Aged skin is thinner, and less reactive than skin in maturity, and it seemed likely that topical corticosteroids would have less effect in an older age group. In this study, four groups of normal male volunteers (aged 18–30, 31–50, 51–64 and 64+ years), were studied in an experiment in which 0·1% betamethasone valerate ointment, 0·005% fluticasone propionate ointment, and their respective bases were applied occlusively three times per week for 3 weeks to flexor forearm sites. Measurements of skin thickness were made by pulsed A-scan ultrasound before, during and after the topical applications, and biopsies from the treated sites were assessed histometrically using an image analysis technique at the end of the experiment.

TABLE 1

Age group (years)	18–30 (*n* = 12)	31–50 (*n* = 12)	51–64 (*n* = 12)	65+ (*n* = 10)
Skin thickness, % Atrophy				
Steroids (combined)	15·7 ± 6·6	14·8 ± 7·5	13·9 ± 8·3	14·7 ± 6·1
Epidermal thickness (μm)				
Bases (combined)	69·0 ± 4·8	62·8 ± 10·0	57·0 ± 12·0	50·8 ± 9·1
Steroids (combined)	46·9 ± 6·9	43·3 ± 9·6	48·4 ± 11·8	47·4 ± 8·3

The results shown that skin thinning occurred with both of the corticosteroids compared to the bases, and that this was similar in all age groups (Table 1). However, epidermal thinning, although similar for the two corticosteroids, was greater in the younger group (Table 1). Other epidermal parameters examined showed no differential corticosteroid effects with age.

In conclusion, it seems that at least some of the effects of corticosteroids on skin may be age-dependent, and this fact may need to be taken into account during treatment.

A clinico-microbiological study of toxic erythemas in children

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A child with an acute febrile illness and 'non-specific' widespread erythematous rash of short duration is a common paediatric problem. This was a prospective study of 100 children presenting to a busy casualty department of a children's hospital over a 1-year period. Children with a clearly recognizable rash (such as classical chickenpox and measles) were excluded.

Details of the clinical presentation were recorded and specimens taken included throat swabs for bacterial and viral culture, urine and stool samples for virology and blood for monospot test, ASO, mycoplasma and viral titres. At 1 month follow up, repeat serology was performed if the infection remained undiagnosed.

In 65 children an infectious agent was identified; 47 were viruses, 13 were bacteria, three *Mycoplasma pneumoniae* and in two children both bacteria and viruses were detected. The most common infections were an atypical presentation of measles and those due to group A beta haemolytic *Streptococci* and picornaviruses.

We would recommend that routine management should include a throat swab for bacterial investigations and in selected cases (where there is a risk to a pregnant woman or to immunocompromised individuals) a blood sample for IgM viral screen.

Response rates to current treatment of common warts and evaluation of continued cryotherapy and inosine pranobex for treatment failures

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Viral warts of the hands and feet represent a substantial proportion, *c.* 15% of dermatological referrals. Cryotherapy is a standard treatment of these warts in this country. The treatment represents a significant drain on doctor and patient time, involving multiple visits for the application of liquid nitrogen. Often additional time is spent on paring. The reported cure rate at 3 months is 75% for hand warts and there is some evidence that a combination of cryotherapy and topical treatment may improve the cure rate further. There is little known about the cure rate beyond 3 months. The immunomodulator, inosine pranobex, has been reported to increase efficacy of physical destructive procedures in clearing genital warts. It has been our impression that 3-month cure rates are less than that above, and that a significant proportion of patients are subjected to long-term cryotherapy.

The aims of the present study were to estimate the cure rate at 3 months from what is currently considered to be an optimal treatment, to investigate the value of paring of the warts and to investigate in the 3-month treatment failures the value of further cryotherapy and the use of inosine pranobex.

Four hundred consecutive referrals with hand and/or plantar warts were enrolled. For the first 3 months all were treated with a combination of 15% salicyclic acid with 10% lactic acid (Salactol), combined with liquid nitrogen application at 3-weekly intervals. Individuals with warts greater than 4 mm were allocated randomly to paring and non-paring groups. Patients who failed to respond at 3 months continued to use Salactol and were randomly assigned to receive further liquid nitrogen or not, and to receive inosine pranobex or matched placebo.

The 3-month cure rate was only 42% for hand warts and 49% for plantar warts. Paring improved the cure rate in the feet ($P < 0{\cdot}025$), but not the hands. Of the 3-month treatment failures, the overall cure rate during the next 3 months was 31%. The cure rate was greatest in the group receiving the combination of liquid nitrogen and inosine pranobex, but this did not reach statistical significance. There was no obvious value in continuing liquid nitrogen during this period.

A serological test for rapid diagnosis of cutaneous tuberculosis

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A serological test based on antigen capture ELISA has been developed for the detection of mycobacterial antigen in circulating immune complexes of patients with active tuberculosis. Preliminary studies indicate a high sensitivity of the test with 80 cases of sputum-positive pulmonary tuberculosis all yielding a positive result and over 100 healthy European volunteers showing a negative result. The test also has high specificity, with negative results obtained in patients with cystic fibrosis, bronchopneumonia, streptococcal throat infection, rheumatoid arthritis, systemic and cutaneous sarcoidosis and psoriasis. The result of the test can be made available within 24 h and the test is much cheaper than the conventional tissue culture method for tuberculosis.

The antigen test was used to make a rapid and reliable diagnosis of lupus vulgaris, scrofuloderma, warty tuberculosis and erythema induratum. The test was found to be positive for infections caused by mycobacterium tuberculosis and mycobacterium bovis, but negative for mycobacterium marinum. After commencing anti-tuberculous chemotherapy, serial tests performed at 4–6 weekly intervals showed an initial rise in the antigen titre followed by a gradual decline. Anti-tuberculous therapy was continued until the antigen titre had fallen into the normal range. Thus, in addition to offering a means to a rapid diagnosis of cutaneous tuberculosis, serial measurements can be used to monitor the effectiveness of anti-tuberculous therapy, to assess compliance, and to determine the optimum duration of therapy.

Dermatological audit: facts or friction

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An audit of out-patient dermatological services in the south-east of Scotland was carried out by medical staff in the Department of Dermatology in Edinburgh during the month of November 1981[1]. An identical study was repeated in November 1988 to gather information about changes in number and pattern of referrals over the 7-year period, and to assess possible effects of recent publicity campaigns aimed at increasing public awareness about skin cancer.

During November 1988, 1592 new patients and 2037 return patients were seen. This represented an increase of 29·2% and 28·5%, respectively, since 1981. The population increase for the same period was 1·5%. The most striking changes in diagnostic groups seen were a 173% rise in new cases presenting with benign tumours (excluding viral warts) and a 106% increase in new patients with malignant tumours. Eight cases of malignant melanoma were seen compared with three in 1981. Viral warts and eczema were, as in 1981, the second and third most common diagnostic categories in new patients. There was a 98% increase in the number of surgical procedures performed on new patients compared with 1981. We conclude that the substantial increase in numbers of both benign and malignant tumours and the consequent increase in surgical treatments was probably a result of increased public awareness and concern about skin cancer.

Whatever the cause of this inundation, if the findings are representative then they carry important implications for the staffing and resource allocation for every dermatology department in the U.K.

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Chloracne: report of an outbreak with new clinical observations

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The lesions of chloracne are morphologically similar to those of acne vulgaris but there are major clinical and pathological differences between the two conditions. We have reviewed 17 recent cases of occupational chloracne with an emphasis on the clinicopathological correlations.

All affected individuals developed the fine comedones characteristic of chloracne. These were most frequent on the face (88%) and forearms (47%), forming extensive sheets in some cases. Comedones occurred also in less conspicuous sites including the back (35%), thighs and buttocks (29%) and genitalia (6%). Cystic lesions were less common (29%), and occurred usually on the face or neck. Several subjects also developed yellowish follicular papules particularly on the neck. Over half those affected (59%) had cutaneous xerosis. This involved mainly the lower limbs, but was extensive in four cases. Areas of folliculitis developed in 53% usually on the back and legs. Involved follicles were typically surrounded by a collarette of scale; the hair shaft was often broken and coiled. Histologically, there was follicular plugging and lymphocyte infiltration. In some areas of destruction of follicular walls, a giant cell infiltrate was present.

The cutaneous xerosis in these patients results from chemically induced inflammatory damage to sebaceous glands. In contrast, the pathogenesis of acne vulgaris lesions involves excessive sebum excretion. This difference may account for the relative ineffectiveness in chloracne of standard therapeutic measures for acne vulgaris. However, slow spontaneous improvement of the acneiform lesions occurs after cessation of exposure to causative chemicals.

Practical use of a disability index in the routine management of acne

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The use of lengthy disability questionnaires is impractical in routine clinics. We have therefore created a simplified questionnaire of five multiple choice questions to assess patient disability in acne. The response was graded 0 (normal) to 15 (most disabled). Sixty-six newly referred patients with acne, 40 male, aged 13–53 (median 21·5) years, completed the questionnaire without supervision; acne severity was graded clinically by comparison with photographic scales. Both were repeated at follow-up visits. At presentation the range of acne severity (cumulative: face + chest + back) was from 0·1 to 10·5 (mean 3·2). The range of disability score was from 2 to 14 (mean 6·5). Overall, there was no significant correlation between acne severity and disability score, although one question strongly correlated with the severity of acne on the back. Twenty-three patients did not attend after the first visit. Twenty-two patients (15 male) were treated with antibiotics and were followed for 1–7 (mean 3·8) months. During this period the mean cumulative acne severity remained at 1·8, but the mean disability score fell from 5·2 to 1·8. Sixteen patients (nine male) were treated with isotretinoin and followed for a period of 2–9 (mean 5) months. During this period the mean cumulative acne severity fell from 2·0 to 0·4 and the mean disability score fell from 5·2 to 3·7. One patient was identified as dysmorphophobic on the basis of a mean acne severity of 0 and a disability score of 12. This short questionnaire adds a patient-orientated dimension to the routine assessment of acne severity and may identify patients with higher levels of disability.

Isotretinoin, creatine kinase and exercise

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We have studied the effect of isotretinoin 1 mg/kg/day on serum creatine kinase (CK), 24-h urinary ceatine excretion, and myalgia, in relation to exercise profile in 26 patients (21 male, 5 female, mean age 22 years, range 16–34 years). The indication for therapy was severe acne ($n = 25$) or hidradenitis suppurativa ($n = 1$). Patients were assessed on two occasions prior to (weeks − 1, 0), monthly during treatment (weeks 4, 8, 12, 16), and monthly after (weeks + 4, + 8) stopping isotretinoin.

Median (1), range (2) CK values (nr 10–171 U/l), and number of raised CK values (3) are shown in Table 1.

TABLE 1

Week	− 1	0	4	8	12	16	+ 4	+ 8
(1)	119	130	120	123	115	116	124	129
(2)	68–845	56–225	61–327	65–443	52–613	58–295	61–316	27–318
(3)	5	7	6	5	7	5	4	7

A raised CK value was observed in 16 (61·5%) patients during the study. The major determinant of the CK value was the patient's current (within previous 6 days) exercise level ($P < 0·01$). Neither background exercise level ($P = 0·09$) nor isotretinoin therapy ($P = 0·48$) had an effect on CK levels.

The CK level was not significantly related to myalgia ($P = 0{\cdot}53$), this symptom occurring in only 13% of occasions when the CK was raised. Myalgia was, however, related to isotretinoin therapy, being present in 28% of patient assessments during, and only 4% outwith the treatment period. There was no significant myoglobinaemia or myoglobinuria at week 0 or at week 16. The mean 24-h urinary creatinine excretion at week 16, before and after correction for creatinine excretion, showed no significant change from week 0.

We conclude that the main determinant of an increased serum CK in patients on isotretinoin therapy is their current exercise level.

Effect of isotretinoin on epidermal proliferation

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Isotretinoin has been used in the treatment of non-melanoma skin cancer, and retinoids have been shown to modulate the epidermal growth factor receptor (EGFr). In this study we have investigated the effects of isotretinoin on EGFr expression and on parameters of epidermal proliferation in normal skin.

Twelve patients (eight males, four females) with acne treated with isotretinoin 1 mg/kg, had a 4-mm punch biopsy taken from forearm skin before treatment, after 1–2 weeks, and after 16 weeks. Portions of the biopsies were studied for (i) epidermal thickness using image analysis, (ii) epidermal cell production using a tritiated thymidine (^{3}HT) autoradiographic technique and an immunocytochemical method based on the uptake of bromodeoxyuridine (BrdU), and (iii) EGFr expression using a monoclonal antibody with intensity of immunoperoxidase staining assessed using a 10-cm visual analogue scale (Table 1).

TABLE 1

Parameter	Result Week 0	 Week 2	 Week 16
MET (μm)	50·1 ± 6·9	54·1 ± 7·9	63·0 ± 5·0*
^{3}HT labelling index (%)	7·2 ± 1·4	8·3 ± 2·0	6·8 ± 0·8
BrdU labelling index (%)	4·5 ± 0·9	6·1 ± 1·6*	3·7 ± 0·9*
EGFr expression (cm)	4·4 ± 0·9	5·1 ± 1·3	5·2 ± 0·9

*$P < 0{\cdot}05$.

There was a statistically significant increase in mean epidermal thickness (MET) after 16 weeks. The BrdU technique showed a significant early increase in proliferative rate followed by a decrease. The ^{3}HT method showed a similar trend but was not statistically significant, using non-parametric statistical tests. The EGFr expression did not change with treatment.

The increase in MET with isotretinoin parallels increases observed with etretinate, as does the alteration in proliferative profile. Our results suggest that these changes may not be mediated through modulation of EGFr expression.

Allergic contact dermatitis reactions to latex examination gloves

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Forty-two patients referred to the Contact Dermatitis Investigation unit for investigation of hand dermatitis were patch tested with the standard European battery and our glove battery. Our glove battery contains 16 different makes of examination gloves used commonly in the Glasgow hospitals: 12 of the gloves are latex, one synthetic rubber, two vinyl and one polythene.

Of the 42 patients, 30 were medical or dental personnel whilst the remaining 12 had a miscellany of occupations which required them to wear such gloves for protection. Eight of the medical or dental personnel reacted to one or more of the latex gloves and, of these patients, seven were also found to be rubber sensitive. Five of those with various occupations reacted to one or more of the latex gloves and of these patients, all except one were found to be sensitive to rubber.

The wearing of latex examination gloves by medical and dental personnel has increased in order to reduce the risk of contraction of the human immunodeficiency virus. Hand dermatitis may be induced in those who are sensitive to rubber, and this may increase the risk of virus transmission to those individuals if their skin is broken and fissured. Rubber-sensitive persons working in at-risk occupations should ensure that there is a ready supply of non-latex examination gloves available.

Contact sensitivity to topical corticosteroids

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Contact sensitivity to topical corticosteroids is recognized but generally considered to be rare. Between July 1987 and November 1989, 4089 patients were patch tested with topical corticosteroids including tixocortol pivalate (TP), hydrocortisone (HC), clobetasol propionate (CP), budesonide, and others. TP is structurally similar to HC and has been suggested as a useful marker of steroid sensitivity.

Forty-seven (1·4%) of patients tested to TP had positive patch tests (PTs); 13 of these (28%) were also positive to HC; seven of the 47 (15%) were positive to CP. Fifteen of the 47 (32%) were positive to one or more other steroids. Seven patients had positive PTs to HC but were negative to TP; 15 had positive PTs to non-HC steroids and were negative to TP.

Twenty-one (1·5%) of patients tested to CP were positive to it and 14 of these (67%) were also positive to one or more other steroids, the commonest being HC ($n=9$, 43%), and TP ($n=8$, 38%)

Of 144 consecutive, unselected patients attending the PT clinic in November and December 1989, 10 (14%) were positive to one or more steroids. The commonest positive PTs were to TP ($n=8$, 5%), HC ($n=5$, 3%), and budesonide ($n=3$, 2%). Budesonide is of particular interest because this steroid has not been available in Britain until recently and these positive tests cannot be explained by clinical exposure.

Tixocortal pivalate is a marker for hydrocortisone sensitivity

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Tixocortol pivalate (TP) has been suggested as a marker for topical steroid sensitivity and it has been recommended that it should be part of the standard series. Unfortunately, its manufacturers will no longer supply the pure compound for patch testing. We have, therefore, obtained the commercial product Pivalone, used for allergic rhinitis in continental Europe and containing TP (1% aqueous solution), and have added it to our standard series. We report our experience.

Between June and December 1989, 195 patients were patch tested to Pivalone, 15 (7·7%) had positive reactions. Positive patch tests were confirmed in these 15 patients by patch testing with pure TP (1% pet) obtained from Dr Dooms-Goossens (Belgium). Previous studies have reported incidences of TP positives ranging between 0·2 and 3·4%. Seven of our patients have also been patch tested to a series of other steroid compounds including hydrocortisone with negative results.

We believe that TP is a marker for hydrocortisone sensitivity rather than for other steroid compounds as it has a similar structure to hydrocortisone; our patients' eczema worsened whilst using hydrocortisone preparations or they had positive usage tests with hydrocortisone ointment. We strongly recommend that Pivalone be on the standard series.

The significance of a positive patch test to mercury in oral disease

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The role of allergy to mercury in the pathogenesis of oral disease remains controversial. During the period 1983–88 we studied 372 patients with a variety of intra-oral conditions including lichen planus, burning mouth syndrome, orofacial granulomatosis and recurrent aphthous stomatitis, by patch testing with the standard European, dental (Chemotechnique), organic mercurial and food batteries.

There were 40 positive reactions to mercury in 29 patients: ammoniated mercury (22 patients), phenylmercuric acetate (nine), phenylmercuric nitrate (one) and thiomersal (eight). In addition, two patients had an irritant reaction to ammoniated mercury. Of these 29 patients, seven complained of burning mouth syndrome, 16 had biopsy proven oral lichen planus, one had generalised gingivitis, and five patients had recurrent aphthous stomatitis. Allergy to mercury was thought to be relevant in 16 patients (14 patients with lichen planus, one patient with recurrent aphthous stomatitis and in one patient with gingivitis), not relevant in 12 (seven burning mouth, three recurrent aphthous stomatitis and two lichen planus) and relevance was undetermined in one patient with recurrent aphthous stomatitis. The 14 patients with lichen planus, in whom allergy to mercury was considered relevant, improved significantly following replacement of their amalgams or following avoidance of contact lens fluid containing thiomersal. The 12 patients in whom the positive mercury reaction was considered not relevant, either did not improve following amalgam replacement or they were edentulous.

The role of allergy to mercury in the pathogenesis of oral disease remains unclear but the results of this study suggest that it plays some part in the evolution of oral lichen planus and should be considered as a possibility in patients with recurrent aphthous stomatitis when this is immediately adjacent to amalgam fillings.

Patch testing in lichenoid reactions of the mouth and oral lichen planus

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The role of allergic contact dermatitis in the pathogenesis of oral lichen planus/lichenoid reactions remains controversial. The standard European, dental (Chemotechnique), organic mercurial and food batteries were applied to 53 patients with biopsy proven, symptomatic oral lichen planus (or lichenoid reactions in the mouth) between 1983 and 1988.

There were 91 positive reactions in 39 patients: mercury/organic mercurials (22 positives in 15 patients), fragrances (20 reactions in 13 patients), foods (12 reactions in 10 patients: eight cinnamaldehyde, four benzoic acid), medicaments (11 positives in seven patients), metals (19 positives in 17 patients: nine $CuNO_3$, six nickel, four other), rubber mixes (four reactions in four patients), and colophony (three patients). In addition, there were several irritant reactions, mostly to mercury and $CuNO_3$. The patch test reactions were thought to be relevant in 21 patients, not relevant in 10 and undetermined in eight patients. Of the 21 patients with relevant positive reactions, 13 were thought primarily to be due to mercury (seven patients had amalgams replaced and all improved, three patients declined replacement and three were lost to follow-up), four to cinnamaldehyde/benzoic acid (all improved on dietary restriction/change in toothpaste), two to rubber mixes (both improved on replacement of rubber pillows), one to nickel (patient with rheumatoid arthritis on gold injections who flared after each injection) and one to formaldehyde (improved after change in toothpaste).

Although the aetiology of oral lichen planus is still unknown the results of the present study would support the view that contact allergy plays a significant role in its pathogenesis.

Pruritus vulvae: a manifestation of contact hypersensitivity?

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The cause of pruritus vulvae (PV) often remains unclear and thus optimal treatment may not always be given. Patients often give a history of long-term use of many topical treatments, which led us to consider that PV may sometimes be related to contact hypersensitivity.

We have assessed the value of patch testing in a group of 50 women (mean age 43·9, range 20–76 years) in whom PV was a major symptom. All were patch tested to a standard battery of agents plus other substances as indicated. Patch tests were read at 48 and 96 h. Details were obtained of the type, onset and duration of symptoms, current and past treatments, personal and family histories of other skin or atopic disease.

Thirty-nine patients (78%) showed at least one positive reaction (group A). Patch tests were negative in 11 individuals (group B). In group A, 19 individuals reacted to one allergen, nine to two, seven to three, and four to four or more. Twenty patients gave a positive reaction to medicaments, i.e. antibacterials, antiseptics, preservatives and local anaesthetics. The most commonly encountered allergens were nickel (14/39), perfumes (13/39), neomycin (11/39) and local anaesthetics (9/39). Nickel sensitivity was only found in pre- or peri-menopausal patients. Atopy was more common in group A than group B patients.

All patients were sent a postal questionnaire on their symptoms at least 1 year after patch testing, 32 (27 group A, 5 group B) replied and of these three (2A, 1B) were asymptomatic, 14 (11A, 3B) had improved, 13 (12A, 1B) were unchanged and two (2A) were worse. Group A patients had been advised on avoidance of their allergen(s) both verbally and by typed information sheets. Twenty-three of group A had tried to avoid their allergens and 16 felt that this had improved their symptoms.

We have examined the value of patch testing in patients with PV: 78% of patients showed positive reactions especially to medicaments and to nickel. In this patch-test positive group avoidance of allergens may result in a lessening of symptoms.

Loss of lanolin patch test positivity

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Once contact sensitivity has been demonstrated, patients can relatively easily avoid lanolin and thereby minimize further stimulation of the immune response. We therefore used repeat patch test response to lanolin to investigate persistence of allergic contact dermatitis.

Thirty-seven adult out-patients who had previously demonstrated a positive patch test to lanolin (30% wool alcohol) more than 6 months previously were rechallenged. The group comprised 11 males and 26 females with a mean age of 45 (range 21–64 years). The mean period since demonstrating a positive patch test to lanolin was 25 (range 7–57) months. Persistence of allergy was assessed by repeat patch testing to lanolin.

Only 41% of the whole group retained their positive patch-test response. The six patients whose retesting was performed within 1 year showed a significantly higher rate of persisting lanolin patch-test positivity at 67%, compared with just 35% of those retested after at least 1 year.

The absence of a positive response to lanolin in 65% of patients retested after 1 year raises questions concerning the long-term significance of an isolated positive patch test to lanolin in particular, and for allergic contact dermatitis in general.

A clinical classification and grading system of the changes in onchocerciasis and histopathological findings

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Onchocerciasis is one of the most important ocular and dermatological diseases of the tropical world. We have developed a classification and grading system for recording the cutaneous changes in onchocerciasis. It is hoped that this could be used as a standard method of description to convey clinical information to other workers and assist immunological studies. The classification scheme was field-tested within the context of a large-scale trial in Northern Nigeria which is assessing a new microfilaricide, ivermectin.[1] Seven thousand pre-dosing skin examinations were studied using this classification and we found that the system has several advantages: it is simple to understand and utilize in the field; furthermore, since it is designed for computer coding, it is suitable for use in other large-scale surveys. The main categories include acute and papular onchodermatitis, lichenified onchodermatitis, atrophy and depigmentation. These were correlated with the histopathological changes seen in 200 punch biopsies. Patients with clinically normal skin generally had multiple dermal microfilariae and significant perivascular infiltration of mononuclear cells. Acute papular lesions are characterized by inflammatory changes near the dermo-epidermal junction. With more chronic forms of disease there is a dense perivascular infiltrate with few parasites.

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Increased risk of cutaneous reactions to Fansidar in HIV disease

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Adverse reactions to drugs, especially the sulpha drugs, have been reported to occur with greater frequency in HIV positive patients.[1] Prolonged survival in HIV disease depends upon effective treatment and prophylaxis against opportunistic infections. Fansidar (pyremethamine 25 mg and sulphadoxime 500 mg) is one of the prophylactic agents used against *Pneumocystis carinii* pneumonia. A survey of HIV-positive patients attending our clinic was undertaken to estimate the risk of an adverse reaction to Fansidar.

The case notes of 551 patients (409 alive, 142 dead) were reviewed. Any possible cases were assessed by a dermatologist. Pharmacy's records and the case notes gave information on those who had taken Fansidar. At least 155 patients had been prescribed Fansidar. There were four definite cases of adverse cutaneous reactions seen by a dermatologist: two had Stevens–Johnson syndrome and had to be hospitalized; two had toxic erythema. None were fatal and all occurred within 3 weeks of starting treatment (one tablet weekly). All patients had low T4 counts (5–114 cells/mm^3) and all had circulating p24 antigen. The risk of a reaction was not associated with other sequelae of HIV disease, atopy or a family history of atopy.

The risk of an adverse reaction to Fansidar in HIV patients appears to be of the order of 1 in 40 compared to an incidence of 1 in 8000 in non-HIV people.[2]

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HTLV-1 associated cutaneous disease

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The clinicopathological features of six patients, who presented with HTLV-1 associated cutaneous disease, are reviewed. All (one female, five males, mean age 45 years) were of West Indian extraction and permanent U.K. residents. Cutaneous features included localized papules (two), a generalized papulo-nodular eruption (three) and plaques and tumours (one). Five sero-positive patients developed leukaemic involvement after a mean duration of 67 months (range 3 months–21 years) and all but one died subsequently, with a mean survival time of 7 months. The cutaneous histology was variable. Most lesions were characterized by a heavy dermal infiltrate composed of abnormal cells and small lymphocytes, histiocytic cells, plasma cells and eosinophils. Epidermotropism was present in three patients. Tumour cells extended between collagen bundles ('Indian filing') and had abundant ambiphilic cytoplasm and large dense pleomorphic nuclei. In one patient these large atypical cells produced an appearance simulating granuloma formulation. Molecular studies performed on tissue DNA from five patients revealed a monoclonal T-cell population, associated with clonal integration of HTLV-1 proviral DNA in three, and in two patients HTLV-1 integration within DNA from lesional skin was established retrospectively using enzymatic *in-vitro* amplification of a specific HTLV-1 pol gene sequence.

This review indicates that the cutaneous prodrome of HTLV-1 associated ATLL can be extremely prolonged. Although rare in the U.K. there is a characteristic spectrum of clinical and pathological features. Molecular analysis of tissue DNA represents a definitive method for establishing an early diagnosis.

Nail-blanching: a new cutaneous sign indicating mechanical and functional impairment of the finger pulp

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A new cutaneous sign is described based upon the personal observation of 13 cases.

Extension of the fingers in the normal hand produces little alteration in the colour of the nail bed or, at most, pallor confined to the distal zone of the nail bed (13 age-, sex-matched controls). When the nail-blanching sign is present the nail bed is blanched of colour over most of its surface area when viewed with the fingers in extension. The sign disappears when the same fingers are viewed in the position of flexion. When the sign is strongly positive the nail is often overcurved in a longitudinal direction producing pseudo-clubbing.

The sign is seen most frequently in atopic hand eczema affecting the fingers, but identical changes can also be seen in hyperkeratotic psoriasis and in some cases of keratoderma. The mechanism of action is believed to be due to rigidity of the digital skin due to hyperkeratotic dermatoses. Other contributory factors are to be discussed.

Patients showing the blanching sign have difficulty in picking up fine objects. They are also a group at risk as their skin is liable to develop painful fissures unless appropriate remedial action is taken promptly. The pseudo-clubbing associated with the nail-blanching sign can easily be distinguished from classical clubbing by the presence of associated cutaneous signs.

Successful treatment of chronic tinea pedis and tinea manuum with Lamisil® (terbinafine)

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In this study Lamisil® (terbinafine) has been compared to placebo in patients with moccasin type tinea pedis or tinea manuum. Lamisil® was given at 250 mg/day for 2 weeks. Patients were enrolled in a randomized, double-blind study. Patients were evaluated weekly by mycological culture, microscopy, clinical signs and symptoms for 2 weeks during therapy and during a 6-week follow-up period. Twenty-eight of the 53 patients were evaluated for efficacy. Twelve out of 14 patients were mycologically cured (86%) at 8 weeks, compared to one out of 14 in the placebo group (7%) (Fisher's exact test, one-sided, $P < 0{\cdot}01$). The number who achieved mycological and clinical cure was less, 10 out of 14 (71%). However, 87% of the cases in the treatment group were due to *Trichophyton rubrum* of whom 43% had already been treated with other agents and the infection had recurred. The safety profile of Lamisil® was satisfactory with eight new symptoms in the Lamisil® group compared to 14 in the placebo group. There were no significant changes in the haematological, hepatic or renal blood tests done weekly.

Oral Lamisil® was found to be safe and highly effective in the treatment of moccasin tinea pedis and tinea manuum when given for a short course of 2 weeks.

Nail matrix kinetics of systemic terbinafine in onychomycosis

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Oral terbinafine (Lamisil®) is very effective in the treatment of toe-nail dermatophyte onychomycosis when given for 48 weeks. The purpose of this study was to directly measure nail-plate terbinafine kinetics, as short courses of therapy might be considered if it could be demonstrated that adequate nail concentrations of the drug were reached at an early stage.

Twelve patients with dermatophyte onychomycosis were studied, taking terbinafine 250 mg daily for 48 weeks (10 patients) and 24 weeks (two patients), as part of a wider multicentre clinical trial. Nail growth was measured. Distal nail clippings were taken from an infected 'target' nail, and from two normal nails every 4 weeks for mycology and drug estimation. Terbinafine was measured in the weighed nail samples using a new HPLC-based technique.

Affected target nails initially grew *Trichophyton rubrum* (eight patients), *E. floccosum* (one patient), *T. mentagrophytes* (three patients). Terbinafine was first detected in nail at a mean of 7·8 weeks (SD ± 4·4, range 3–18 weeks) after starting therapy and nails became culture negative at a mean of 7·3 weeks (SD ± 9·9, range 0–32 weeks). However, there was no correlation between time to becoming culture negative and time to first detection of terbinafine in individual patients. Nails became negative for fungi on microscopy at a mean of 29·7 weeks (SD ± 15·6, range 0–48 weeks) after starting therapy. Mean rate of nail growth in target nails was 0·41 mm/week (SD ± 0·17, range 0·3–0·8). Mean terbinafine levels were within the range of 0·25–0·55 ng/mg of nail throughout the study and did not progressively increase.

The kinetic profile of terbinafine in normal nails was very similar to that in infected nails. This study shows that terbinafine diffuses into nail plate rapidly and suggests that much shorter courses of terbinafine may be effective in treating onychomycosis.

A follow-up study of terbinafine (SF-327, Lamisil®) in the treatment of onychomycosis

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Current therapy of onychomycosis is disappointing, and relapse rates after treatment are high. Terbinafine (SF-327, Lamisil®) is a novel orally active synthetic fungicidal agent of the allylamine class. We have previously reported the successful use of this drug, but follow-up was limited. We now report a follow-up study.

Of 103 patients with culture-positive finger-nail (FN) and toe-nail (TN) dermatophyte infection treated with oral terbinafine 250 mg daily for up to 6 (FN) and 12 (TN) months, 95% of 21 patients with finger-nail and 85% of 62 with toe-nail infection achieved mycological cure (negative microscopy and culture at last assessment). Only seven patients withdrew due to adverse events, six of whom suffered dyspepsia.

Fifty-nine of the 73 patients with mycological cure have undergone follow-up assessment for up to 12 months after treatment; five (8%) have clinical signs of relapse. Only one of 36 patients followed for a full 12 months has mycological recurrence. Five of 10 patients recorded as failures of therapy had achieved clinical cure when reviewed. The mean rate of appearance of unaffected toe-nail was 1·0 mm/month. There were significant differences in this rate between those with toe-nail infection successfully treated (> 1 mm/month from the commencement of treatment) and failures (< 1 mm/month until fourth month of therapy). The extent of infection increased initially in the latter group.

Oral terbinafine is an effective and well-tolerated treatment for onychomycosis. The rate of appearance of uninvolved nail over the first 4 months of treatment is a useful indicator of cure. Follow-up data indicate a low rate of relapse.

Abnormal lymphatic function assessed by quantitative lymphoscintigraphy in yellow nail syndrome

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Oedema is a frequent finding in yellow nail syndrome (YNS) and has long been considered to be lymphoedema on the basis of abnormalities demonstrated by contrast X-ray lymphangiography.[1] Isotope lymphoscintigraphy (IL) provides an objective measurement of limb lymphatic function and reliably distinguishes between lymphoedema and oedema of non-lymphatic origin.[2] Patients with YNS were investigated by IL in order to evaluate limb lymph drainage and to establish if any abnormality of lymphatic function exists. Five patients (four male, one female) with classical YNS were studied, their ages ranging from 13 to 76 (mean 55) years and duration of disease ranging from 5 to 25 (mean 12) years.

^{99m}Tc-antimony colloid was injected subcutaneously into the first web space of each foot and each hand. Upper and lower limbs were examined sequentially. Percentage uptake of isotope colloid in the inguinal and axillary lymph nodes at 2 h was taken as an index of lymph drainage. Normal nodal uptake was expressed as a percentage of the administered dose.

Results demonstrated reduced nodal uptake in patients with YNS compared to normal controls. Inguinal node uptake (± SEM) in normal subjects was 17·72% (± 1·82, $n = 14$) compared to 8·5% (± 2·02, $n = 10$) in YNS. Axillary node uptake (± SEM) for normal subjects was 8·55% (± 2·3, $n = 4$) compared to 5·5% (± 1·17, $n = 10$) in YNS. The results in YNS, although lower than normal, do not approach the low values seen in chronic lymphoedema. Taken with the fact that the clinical appearance of YNS oedema does not conform to that seen in lymphoedema, these results suggest that the oedema of YNS is not solely lymphatic in origin and not therefore, by definition, lymphoedema. The possibility, however, of oedema of mixed origin with some lymphatic insufficiency cannot be excluded.

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Abnormal lymph drainage in patients with chronic venous leg ulcers

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Chronic venous leg ulcers afflict approximately 1% of the U.K. population and are a major drain on resources. One possible factor responsible for the poor rate of healing of venous leg ulcers is oedema which is a common association. The efficiency of lymph drainage in patients with chronic venous leg ulcers has been evaluated using isotope lymphography. ^{99m}Tc-antimony colloid is injected into the first web space of each food. The percentage uptake of the tracer in the inguinal nodes at 2 h reliably distinguishes between lymphoedema and oedema of non-lymphatic origin.[1] Twelve patients (two male, ten female; ages 40–86 (mean 70·4) years) with 15 ulcerated limbs underwent isotope lymphanography. If there was bilateral ulceration, the worse affected limb was defined as the leg with the longer history of ulceration.

There was impaired nodal uptake in ulcerated legs ($\text{mean} = 6{\cdot}97 \pm 1{\cdot}02$ SEM, $n = 15$) compared with legs of normal controls ($\text{mean} = 17{\cdot}7 \pm 1{\cdot}82$ SEM, $n = 14$) or legs of patients with oedema and varicose veins ($\text{mean} = 17{\cdot}1 \pm 0{\cdot}91$ SEM, $n = 6$) ($P < 0{\cdot}001$). There was a significant decrease in lymphatic function in the worse affected leg compared to the contralateral one ($P < 0{\cdot}001$). Lymph drainage may be significantly impaired in limbs with chronic venous leg ulcers: this will contribute to the oedema. Oedema discourages wound healing and promotes infection. High external compression is required to treat lymphoedema. These findings may explain the recently reported benefit from high compression bandaging in healing chronic venous ulcers.[2]

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Pathogenesis in bamboo hair

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The pathogenesis of various hair deformities in Netherton's syndrome is unclear.[1,2] Various pathological findings in serial sections (longitudinal and transverse) of 20 deep oval scalp biopsies (eight patients) were correlated with the appearance of hair deformities on polarizing and scanning electron microscope. This allowed the reconstruction of the bamboo deformity. Bamboo hair is caused by transitory inward bulging of the external root (increase of a few cells), pushing the internal root into the keratogenous zone. The direction of the bulge (inward, and also downward), its magnitude (sector-like, discontinuous, circumferential) and the plane (even–uneven) determines the shape of the deformity: one-sided and circumferential invagination, and bamboo hair. In 'bamboo hair' which varies in appearance, serial sections show the downward circumferential wedge-like bulge reducing free passage. Main stream upward moving cells below the constriction assume a zig-zag pattern, weakening the link with the normally orientated cells above and allowing easier breakage and sideways expansion. Peripheral cells are moulded by the lower side of the downward bulge, the whole resulting in the proximal 'cup-like' expansion. Cortical cells present above and before the constriction took place, form the distal 'club-shaped' fragment. A circumferential or other type of bulge occurring in close proximity, not necessarily at the same time, are responsible for unusual looking 'bamboo'.

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Incomplete melanogenesis with melanocyte abnormalities in acute alopecia areata

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It is well-recognized that alopecia areata may preferentially affect pigmented hair, spares white hair, and that regrowing hair in the disease is often initially white. In addition, there is an association with vitiligo and ocular depigmentation. It has been hypothesized that melanocytes may be involved in the pathomechanism of alopecia areata,[1] but no ultrastructural evidence has been documented.

Using light and electron microscopy, 10 patients with untreated acute alopecia areata were studied, examining scalp biopsies from sites with exclamation mark hairs. Control biopsies were taken from three normal scalps.

The controls had the characteristics of normal follicular melanocytes with melanosomes present in all four stages of synchronized pigment deposition.[2] Alopecia areata affected melanocytes showed increased electron density of both nucleus and cytoplasm with 'aborted' melanization of affected melanosomes. Melanosomes presented a variety of bizarre appearances and lacked the normal ellipsoidal shape. Hypertrophy of the Golgi apparatus occurred with distension, disruption and irregular cisternae. Langerhans cells were present in amelanotic bulbs and melanin-laden macrophages were abundant, especially in the dermal components of the follicles. Giant spherical melanin complexes containing up to 100 melanosomes occurred in the hair bulbs of some follicles. An unusual outer root sheath distribution of active melanocytes was present in some cases.

These morphological changes in affected melanocytes support evidence for melanocyte involvement in the pathogenesis of alopecia areata.

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Haemostatic abnormalities in Sneddon's syndrome

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Four female patients with Sneddon's syndrome (livedo reticularis, cerebrovascular lesions and negative antiphospholipid antibodies) were studied. Three had multiple cerebrovascular accidents with infarcts on CAT scan and multiple transient ischaemic attacks (TIAs). One had TIAs only. All had been treated empirically with aspirin, persantin or prednisolone. Primary haemostasis (shear-induced platelet plug formation), coagulation and thrombolysis were measured from non-anticoagulated blood samples using the haemostatometer technique.[1] The pattern of 'bleeding' from holes punched in polythene tubing through which native blood had been perfused, together with subsequent coagulation was monitored by perfusion pressure changes and analysed. Simultaneously, time lapse of expulsion of the haemostatic plugs formed from identical native blood was recorded and considered as spontaneous thrombolysis.

All patients had hyper-reactive platelets, three of the four had grossly enhanced haemostasis and three had hypercoagulation. Two did not have spontaneous thrombolysis, one had prolonged thrombolysis and in one thrombolysis was normal. Patients were commenced on anti-platelet or thrombolytic medication with aspirin, nifedipine or both. Repeated haemostatometer readings were used to monitor dosage and frequency of therapy. Clinical improvement correlated with improvement of haemostatic parameters measured. Patients were followed up for 1–2 years. One patient had two further TIAs but this was an improvement; the other three were asymptomatic.

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Patient information leaflets in the dermatology out-patient department

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Communication between doctor and patient is an essential part of patient care. A questionnaire study was undertaken in the dermatology out-patient department in order to examine patients' opinions of the information provided about their skin problem, and to assess the usefulness of information leaflets.

Follow-up patients were asked to complete a questionnaire before and during an information leaflet campaign. This comprised a constant supply of leaflets and booklets which were displayed in the waiting area for 4 months. Leaflets covered over twenty major relevant topics. The same questionnaire was completed by patients who had been exposed to the campaign on at least one visit. An SPSS computer programme was used for statistical analysis of questionnaires before and during the campaign.

Eight-hundred and fifty-two questionnaires were completed over 1 month before, and 560 during the campaign when 6,630 leaflets were taken. The groups of patients before and during the campaign did not differ significantly in their composition of age, sex, social class, frequency of hospital visits or skin problems. The information in the clinic was rated either 'good' or 'excellent' by 72% of patients. The majority received their information from the hospital doctor (78%) and family doctor (61%). Despite this, 55% of patients felt that the information could be improved, and 63% wanted more time discussing their skin problem with the hospital doctor; 32% wanted the chance to watch a video about their skin problem. The leaflet campaign did not alter these views significantly. Patients less than 60 years of age were less satisfied with the information ($P < 0{\cdot}001$) and 61% wanted an improvement as opposed to 41% of those over 60 ($P < 0{\cdot}001$). Only a small proportion (6%) of patients obtained their information from leaflets, but this increased (20%) following the leaflet campaign ($P < 0{\cdot}001$) and the demand for leaflets was subsequently reduced ($P < 0{\cdot}001$).

Dermatology out-patients have a high expectation of the standard of information which they receive, particularly those who are under 60 years of age. Leaflets in the waiting area are useful for the augmentation of information, but their importance is overshadowed by the patient's perceived need to spend more time discussing his or her skin problem with the hospital doctor. Videos may also be useful for delivering information to the patient.

Infantile systemic hyalinosis: a newly recognized disorder of collagen?

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We describe four infants who presented in the first few months of life with stiff skin and painful joint contractures. Other features included pearly papules particularly on the face and trunk, peri-anal nodules, hyperpigmentation over bony prominences, gingival hyperplasia, persistent diarrhoea and failure to thrive. Two of these infants died before the age of 18 months.

In each case hyaline material was demonstrated in the papillary dermis on light microscopy. Ultrastructural examination revealed a distinctive fibrillo-granular appearance in which a banding pattern could be observed. This material was also found within membrane-bound vacuoles in macrophages and fibroblasts. The appearances and localization are identical with that of collagen type VI. This appears to be a distinctive clinical disorder which may be due to excessive deposition of type VI collagen.

Prenatal diagnosis of genetic skin disease using fetal skin biopsy: 10 years' experience

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The lack of effective treatment for a number of severe genetic skin disorders and increasing awareness that prenatal diagnosis (PND) is available, have led to a growing demand for PND. We now present our cumulative 10-years' experience of PND using foetal skin biopsies obtained at 16–24 weeks' gestation. Multiple samples from each pregnancy were examined by light and electron microscopy. During the past 5 years, additional samples from EB-risk foetuses were also examined by immunofluorescence using specific antibody probes. The total number of pregnancies examined was 83 which includes the following groups. Epidermolysis bullosa (EB): junctional, 42 (10 affected); recessive dystrophic, 13 (2); dominant dystrophic, 2 (1). Ichthyosis: bullous, 3 (1); non-bullous, 10 (0); harlequin, 4 (1); Sjögren-Larsson, 1 (0). Anhidrotic ectodermal dysplasia, 2 (0). Oculocutaneous albinism, 6 (3). Pregnancies at risk for EB were assessed twice in nine patients, three times in four, and four times in one. There was one set of twins (dizygotic) at risk for junctional EB with one twin affected. There was one intra-uterine death and six pre-term deliveries after a 'negative' test. Biopsy scars were usually trivial and difficult to identify. There were no known false negative or false positive results. There was full concordance between two of us (R.E. and B.L.) examining different samples from over 20 EB-risk pregnancies independently. These results strengthen our suggestion of 5 years ago that this type of PND should be offered as a service to couples at risk for certain genodermatoses. The procedure is relatively safe and highly reliable. However, there will always be real or potential difficulties with sampling and analysing foetal skin for this form of PND, even in experienced centres.

Malignant melanoma in Sheffield 1971–88

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Sheffield has a static population, served by a single dermatology clinic where over 95% of all cutaneous malignant melanomas are seen. Over the years we have been able to keep an accurate record of the incidence of melanoma in our city.

Between 1971 and 1988, 411 primary cutaneous malignant melanomas occurred in the city of Sheffield. The incidence rates for all age groups and both sexes have been standardized to the 1971 population census. During this period the incidence rose from 1·75/100,000 for males and 2·7/100,000 for females in 1971 to 7·78/100,000 for males and 10·33/100,000 for females. There was a sharp rise in the last 3 years of the study and the 2:1 female to male ratio usually seen for incidence figures in the U.K. and in the earlier years of the study has narrowed. Incidence rates in the 0–20 and 80+ years age groups have remained static, the rate in the 20–80 age groups rose steadily, though the rise was steeper in the 40–80 age groups in both sexes.

The site of lesions was known in 387 of the 411 patients, the trunk being the commonest site in males and the lower leg in females. The increase in the numbers of melanomas was largely accounted for by lesions at these sites.

With the rising incidence of malignant melanoma, public education campaigns are becoming more important. Our data suggests that much of the increase is occurring in the 40–80 age groups and any campaign to increase public awareness of this condition should be aimed particularly at this section of the population.

Public education for melanoma in Leicestershire: an update

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The Cancer Research Campaign has funded seven centres (including ourselves) for 3 years to undertake melanoma publicity exercises and record the effects on workload and outcome. We have reported the initial effects of the 1987 campaign in the Leicestershire, which showed a statistically significant rise in the number of MMs and a percentage shift towards thin lesions (< 1·5 mm) due largely to an absolute increase in tumours in this category.[1] Two further years have now elapsed and similar education exercises were conducted in the summers of 1988 and 1989. The number of MMs seen in Leicestershire during the second evaluation year remained high at 76 (there were 67 in the first year) and in the period following the week of education there was a percentage shift towards thin tumours similar to that seen in the first year: 60 to 78% in 1986–87; 70 to 77% in 1987–88. In the third year, there was noticeably less media interest in our activities, especially from television stations. The number of new MMs fell to 53, and the percentage of good prognosis MMs returned to levels recorded before the campaign was initiated, although there was a shift towards thin lesions: 59% before and 64% after publicity. These findings suggest that publicity was successful in encouraging the presentation of thin MMs which would not otherwise have attended for treatment until 1989 or later. They also suggest that thought needs to be given to the style and frequency of future melanoma education.

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In vivo measurement of melanin pigmentation

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Non-invasive measurement of melanin pigmentation *in vivo* can provide useful clinical information in a variety of skin disorders. If an objective and easy method of melanin measurement were available then the relationship between skin colour and UVR sensitivity could be characterized. We have designed and constructed a small hand-held meter which produces an index of melanin pigmentation (0 = least, 200 = gretest degree of pigmentation). The probe consists of two solid state monochromatic light sources and a single detector. Measurement of the ratio of diffuse reflectance of skin at the two wavelengths is logarithmically transformed and a constant factor introduced to give a convenient range of units (0–200).

We have compared measurements of melanin index with skin colour assessed by independent observer ranking and Boston classification of skin types, from volunteer subjects of all skin types (Table 1).

TABLE 1

Skin type	Melanin index (mean ± 1 SD)
6	100 ± 43
5	53 ± 19
4	37 ± 6
3	28 ± 8
2	24 ± 3
1	20 ± 2

There was a great deal of variation within each skin type group, as this classification does not explicitly account for actual pigmentation. However, it can be seen that lower skin types had lighter skinned subjects. Visual ranking of actual skin colour did show a very good correlation with the meter reading (Spearman's rank correlation coefficient = 0·88).

We have shown that our meter can give a clinically relevant, easy to understand index related to the degree of pigmentation, which could provide an objective and continuous measure of 'skin type' and enumerate the degree of pigmentation as a clinical sign.

How do malignant melanomas present and does this correlate with the point check list?

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A pigmented lesion clinic was established to coincide with the public education campaign on malignant melanoma. One hundred cases of malignant melanoma have subsequently been seen and evaluated prospectively with regard to the usefulness of the MacKie 7-point check list as a guide to diagnosis. The most frequently reported patient observations were growth and change in colour (especially darkening) and shape. Itching and bleeding were remarked upon in 20% or less. Inflammation was visible in only 11%. Fifty per cent of the lesions were less than the 1-cm size referred to in the check list and 15% were less than 0·5 cm: 73% of our patients had early lesions less than 1·5 mm Breslow thickness and nearly half presented at the insistence of their relatives or friends, or because of the family doctor's incidental diagnosis. They stated they would not otherwise have attended the hospital and many had noticed nothing untoward even on direct questioning. Half of the patients scored less than 3 on the check list and nodular lesions in particular could have been misdiagnosed. The check list has subsequently been revised and now provides a more accurate guide.

The effect of pregnancy on melanoma prognosis

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In the U.K., twice as many women as men develop melanoma and the median age at presentation is 45 years. As 50% of females who develop melanoma do so in their reproductive years, there is increasing concern on the part of both doctor and patient as to whether or not the prognosis for a melanoma patient is changed by pregnancy. In the WHO Programme we have gathered data on 476 patients, 101 of whom have had a melanoma prior to a pregnancy, 110 of whom have developed melanoma while pregnant, 191 developing melanoma after completion of their family, and 74 developing melanoma between pregnancies. At the time of diagnosis 434 patients had stage 1 and 42 patients stage 2 disease; 305 lesions were on the limbs, 124 on the trunk, 32 on the head and neck and 15 elsewhere. There were 32 therapeutic terminations in the entire group.

Breslow thicknesses were 0–1·49 mm for 51%, 1·5–3·49 mm for 26% and over 3·5 mm for 23% of the population. Five-year survival for patients with stage 1 disease were 80% for melanoma before, 66% for melanoma during, 73% for melanoma after and 78% for melanoma between pregnancies. There is a statistically significant difference in survival ($P<0{\cdot}01$) for those whose melanomas developed during pregnancy, but no significant differences between the other groups. There was also a significant difference in survival for patients with melanomas in the middle thickness category, 1·5–3·49 mm thick, with those who developed a melanoma during pregnancy again having a poorer prognosis, but no statistically significant differences in survival between groups in the thin and thick Breslow category. This is the largest study yet carried out on the effect of pregnancy on melanoma: it suggests that melanoma developing during pregnancy carries a slightly worse prognosis, but does not suggest that a pregnancy after recovery from melanoma in any way changes the original prognosis.

The prevalence of solar keratoses in a South Wales population

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Non-melanoma skin cancer (NMSC) and precancerous lesions appear to be increasing in frequency in all Caucasian populations studied. However, the prevalence of these lesions does not appear to have been accurately characterized previously in temperate zones. We have investigated the prevalence of solar keratoses in those over 60 years old in South Glamorgan, which contains a large proportion of individuals of Celtic origin who are acknowledged to be predisposed to NMSC. The subjects (560) were randomly selected from the Regional Family Practitioner Register and were visited at home. All subjects were interviewed and the light-exposed areas on the face, forearms, hands and lower legs were examined. All lesions were photographed and a proportion were biopsied in order to obtain validation of the clinical diagnosis. The prevalence of solar keratoses in the different age groups, and according to sex and skin type, is given in Table 1.

TABLE 1

Variable		Numbers/percentage with keratoses	
Sex	Male	230	32
	Female	329	16
Age (years)	60–64	138	13
	65–69	161	14
	70–74	90	29
	75–79	92	35
	80–84	47	30
	85–89	23	30
	90–94	7	57
	95–99	2	100
Skin type	I	47	43
	II	193	29
	III	237	19
	IV	74	9·5
	V	3	0
	VI	2	0

There was a high degree of concordance with the allotted diagnosis for the non-melanomatous skin lesions photographed between three experienced dermatologists. This study underlines the high frequency of solar keratoses in the elderly population and emphasizes the need for prophylactic measures.

Only cartilage needs to be removed in the surgical treatment of chondrodermatitis nodularis

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This study was designed to investigate the type of surgical procedure necessary for the treatment of chondrodermatitis nodularis (CN): 59 ears in 54 patients (18 female, 36 male), mean age 61 (41–89) years) with CN were studied; five patients had involvement of both ears.

Lesions occurred on the antihelix in 24 (16 female, 8 male), and on the helix in 35 (4 female, 31 male) ears. Symptoms had been present for an average of 18 months and seven patients had been treated surgically before referral. Eleven out of 40 ears improved with intralesional and topical steroids; 29 did not respond. Three patients did not require any treatment, one died before treatment was arranged, and five are waiting for surgery. Operations, under LA, were performed on 39 ears. In all cases a 3-mm punch biopsy was taken through the lesion and the skin incised; on the helix a linear incision was made along the helix and on the antihelix a flap was raised to include the lesion. The underlying area of cartilage was exposed and approximately 10 mm of cartilage on either side of the lesion excised, taking great care to leave no rough cartilage edges before resuturing the skin edges. Twenty-five lesions were treated on the helix and followed up for a mean of 11 (range 1–26) months; small recurrence occurred in four at 1–15 months. On the antihelix 14 operations were performed and followed up for 12 (range 1–31) months. Five have recurred at 5 (range 1–17) months. Recurrences always occurred on the edge of the cartilage excision over a prominent or palpable cartilate edge. Recurrences on the helix did not interfere with sleep, thus no further treatment was required. On the antihelix repeat surgery was tried in three and was successful in one; intralesional steroids were ineffective in three. This study describes a new and successful method for the treatment of CN and shows that only cartilage needs to be removed.

Bath PUVA for psoriasis: the West Midlands experience

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Since the introduction of bath PUVA[1] in the West Midlands in 1986, forty patients with chronic plaque psoriasis have been treated. The concentration of 8-methoxypsoralen (8-MOP) in the bath water was 3·7 mg/l. Twenty-two of 25 patients with skin types I–III receiving bath PUVA achieved clearance of psoriasis at a final and total UVA irradiance of 2·1 ± 0·2 J/cm^2 and 28·3 ± 4·7 J/cm^2, respectively. The comparable values for a control group receiving oral PUVA were 6·0 ± 0·5 J/cm^2 and 110·7 ± 13·8 J/cm^2. Patients with skin types V and VI also improved or cleared on bath PUVA, but required final and total UVA irradiance of 10·0 ± 0·8 J/cm^2 and 215·8 ± 20·2 J/cm^2, respectively. In addition, 10 patients receiving oral PUVA and requiring UVA irradiance greater than 13 J/cm^2 were transferred to bath PUVA at one-quarter of the UVA dosage without loss of control of psoriasis. No systemic side-effect was experienced by patients receiving bath PUVA, and the frequency of pruritus and burning was the same as for oral PUVA.

It is recommended that psoriatic patients receiving oral PUVA should be transferred to bath PUVA if they experience troublesome nausea from oral administration of 8-MOP or require a long exposure time to UVA. Bath PUVA is also more appropriate for patients with skin types V and VI.

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The influence of indomethacin on disease activity in patients with psoriasis during treatment with the Ingram regime

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A number of anecdotal reports have suggested that some non-steroidal anti-inflammatory agents (NSAID) may aggravate psoriasis by inhibition of cyclo-oxygenase and diversion of arachidonic acid metabolism through the alternative lipoxygenase pathway. It has also been suggested that these agents may act beneficially in psoriasis by improving tolerance to dithranol therapy which is often limited by irritant reactions. Previous studies have suffered from small numbers and we have therefore undertaken a large scale study to assess the effect of oral indomethacin in patients with psoriasis treated by the Ingram regime.

Fifty patients with psoriasis undergoing a standard Ingram regime were entered into a double-blind comparison of oral indomethacin 25 mg thrice daily and placebo over a 6-week period. Patients were assessed at 0 and 6 weeks for percentage body surface area involved. In addition, scores on a scale of 0–3 were given for erythema, induration and scaling in two selected plaques. The mean of the sum of scores from each plaque was taken as a severity score for each patient. Forty-one patients completed the study (19 in the indomethacin group and 22 in the placebo group). The two groups were similar in respect of age, sex and severity of psoriasis at entry into the study. There were no significant differences between the indomethacin and placebo groups in respect of percentage reduction in body surface area involved (median 66·7%, interquartile range 31·6–95·7 and median 91·4%, interquartile range 41·2–94·4, respectively) or of percentage reduction in plaque severity scores (median 80%, interquartile range 50–100 and median 82%, interquartile range 55·6–93·3, respectively). The maximum tolerated dithranol concentrations achieved were similar for indomethacin and placebo groups (mean 1·45%, SD 0·72 and mean 1·57, SD 0·66, respectively). Irritancy necessitating a reduction in dithranol concentration occurred in 42·1% and 36·4%, respectively. Four patients in the placebo group (18·5%) and two in the indomethacin group (10·5%) had an increase in skin involvement during the treatment period.

We have shown that oral indomethacin has no significant therapeutic or harmful effect on psoriasis in patients treated with the Ingram regime nor on their tolerance to dithranol. Our observation that worsening of psoriasis occurred in a proportion of both indomethacin and placebo-treated groups calls into question the significance of anecdotal reports of worsening of psoriasis in patients taking NSAID. There are no grounds to withhold indomethacin from patients with psoriasis and co-existent arthritis.

Four years' experience of cyclosporin for psoriasis

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Forty-four patients with severe psoriasis have been treated with cyclosporin (CyA) for 2–50 months (mean 17 months). During the study, 31 (70%) of the 44 achieved > 70% reduction in their PASI score, 39 (88%) > 60% reduction, and 42 (95%) > 50% reduction. The mean initial dose of CyA was 3 mg/kg/day, and the mean dose was 3·3 mg/kg/day throughout the study. Twenty-five (57%) of patients were maintained on 3 mg or less, six patients (14%) required > 5 mg/kg/day for limited periods to obtain significant improvement. In three, this was achieved with 6 mg/kg/day, but in the other three, one required 7 mg and the other two 10 mg/kg/day. Thirty-two (73%) of the 44 patients are still taking CyA. The reasons for discontinuing CyA were side-effects directly attributable to CyA in four; remission of psoriasis in four; one patient has died; one defaulted; one was an infrequent attender, and in one treatment was discontinued because high doses of NSAID were necessary for treatment of his arthritis. Thirty-nine patients were normotensive prior to starting CyA and 21 (54%) developed mild hypertension. In 28 patients who had their glomerular filtration rate (GFR) estimated before and during CyA treatment, there was a 16% reduction ($P > 0\cdot0001$) during a mean period of 8 months of treatment. Two patients developed malignancies. The incidence of hypertension and percentage decrease in the GFR showed a strong correlation with the dose required to control the psoriasis. CyA is an effective treatment for severe psoriasis. However, for long-term maintenance the dose should not exceed 5 mg/kg/day.

A double-blind placebo-controlled trial of topical PUVA in persistent palmoplantar pustulosis

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Persistent palmoplantar pustulosis (PPP) is a chronic disorder which commonly presents a considerable therapeutic problem. Oral psoralen photochemotherapy (PUVA) has been shown to be of value in the treatment of PPP in an open controlled study.[1] Topical administration of psoralens avoids many of the potential disadvantages of oral administration. Uncontrolled studies of topical PUVA in PPP have reported conflicting results. We have investigated the efficacy of topical PUVA in PPP in a double-blind placebo-controlled study.

Patients with bilateral involvement of the palms and/or soles were randomly allocated topical PUVA to one side and a sham procedure to the other. Topical treatments were continued unchanged to both sides throughout the study. Topical PUVA consisted of an application of 0·75% 8-methoxypsoralens in a hydrophilic water/ointment emulsion 10 min prior to UVA exposure three times a week for 8 weeks. The sham procedure consisted of application of the emulsion base alone 10 min prior to exposure to the same light source with insertion of a perspex plate to filter the UVA between the hand/foot and the light source. Assessments of the percentage of surface area involved, pustule counts and severity of erythema, scaling and fissuring on a scale of 0–3 were made at 0, 4 and 8 weeks. Patients' symptoms were assessed on a 10-cm linear analogue scale.

Hand and foot involvement were analysed separately (12 and 15 patients, respectively). There were significant improvements during the treatment period in all assessment parameters except in pustule counts in placebo-treated hands. Topical PUVA produced a significantly greater percentage reduction in area involved than placebo in those with hand involvement at 8 weeks. Significant percentage reductions in area involved and pustule counts were seen in those with foot involvement at 4 weeks but not at 8 weeks. There were no differences between topical PUVA and placebo in patients with hand or foot involvement in respect of erythema, scaling and fissuring scores, nor symptom assessments.

Topical PUVA has some therapeutic effect in PPP. However, the clinical improvement is small and does not result in significant symptomatic improvement compared with placebo. This study indicates that topical PUVA does not produce clinically worthwhile improvement in PPP. Oral PUVA may be a more effective alternative in patients unresponsive to other measures.

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Urticaria pigmentosa: systemic evaluation and successful treatment with topical steroids

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Nine patients with adult onset urticaria pigmentosa were studied for the incidence of extracutaneous mast cell involvement and the efficacy of potent topical corticosteroid therapy for cutaneous lesions. Seven of the nine patients had increased mast cells in the marrow biopsy and five patients had focal aggregates of mast cells. The bone scan was abnormal in one patient. Liver–spleen scans revealed a shift of colloid uptake from liver to spleen in four patients. No abnormal GI radiograms were obtained. Urinary histamine metabolites correlated with bone marrow involvement but not with other parameters. Psychoneurological testing revealed significant deviation from the norm.

All nine patients were treated with 0·05% betamethasone dipropionate ointment under occlusion to one-half of the body nightly for 6 weeks, with excellent response in seven patients. Two patients developed transient abnormalities in HPA axis suppression, both of whom used the medication improperly. Slow return of lesions was noted 6 months after completion of therapy. Remissions could be maintained with single weekly applications of topical steroids.

Systemic involvement is frequent in patients with cutaneous mast cell disease and it is best demonstrated by bone marrow biopsy. Mast cell lesions can be safely and effectively treated with topical steroids in motivated patients.

A histamine releasing factor in serum of chronic urticaria with anti-IgE autoantibody-like properties

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The aetiology of chronic urticaria (CU) is unknown though an allergic cause is often assumed. A serum factor has previously been demonstrated by a wealing response to intradermal autologous serum. We aimed to characterize the serum factor using skin test reactivity *in vivo* and basophil histamine release *in vitro*.

Twenty-four CU patients (17 female, mean age 43·6 years), five patients with symptomatic dermographism (three male, mean age 43·2 years) and five healthy controls (three female, mean age 37·8 years) were recruited. Mean saline-corrected skin test weal volumes ($mm^3 \pm SD$) at 60 min were $38{\cdot}8 \pm 34{\cdot}1$ for CU patients, $2{\cdot}8 \pm 3{\cdot}3$ for dermographics and $-0{\cdot}1 \pm 6{\cdot}7$ for healthy controls. Skin test reactivity of CU serum ultrafiltrates was confined to fractions > 100 kDa ($n = 8$) with little or no response in either control group.

Sera of 9/24 CU patients elicited $> 10\%$ histamine release (HR) above basal values from basophil leucocytes (BL) of one normal donor (mean $49{\cdot}8 \pm 25{\cdot}4\%$) at twofold dilutions. The response varied between basophil donors and was substantially lower in CU BL. The histamine releasing activity was only present in CU serum ultrafiltrate fractions > 100 kDa ($n = 8$). HR in response to CU sera and goat antihuman IgE followed a similar time course and could be abolished by desensitization of BL to A-IgE. HR activity was heat stable (56°C, 2 h, $n = 6$) and was competitively inhibited by preincubation of sera with myeloma IgE. Preliminary protein G affinity column analysis indicated that the HR activity resides in the IgG fraction of at least some patients.

Peripheral blood cellular histamine (ng/ml ± SD) of CU patients ($21{\cdot}5 \pm 12{\cdot}4$) was significantly reduced when compared to healthy controls ($90{\cdot}7 \pm 31{\cdot}7$). BL counts were also reduced in CU patients suggesting that the serum factor causes chronic degranulation. Our results demonstrate a serological histamine releasing factor in CU with properties like an IgE autoantibody which may have an important pathogenetic role *in vivo*.

A histopathological study of 643 cutaneous horns

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A retrospective study of 643 cutaneous horns removed between 1970 and 1989 revealed that 32·8% were derived from either malignant or premalignant lesions. Five parameters influenced the malignant tendency of the base of a cutaneous horn. (a) Age: the mean age of the patients in the premalignant or malignant group was 8·9 years older than that in the benign group (95% confidence interval 5·6–12·6, $P < 0{\cdot}0005$). (b) Sex: male patients were more likely to develop (pre) malignant lesions ($P < 0{\cdot}02$). (c) Site: 70% of all (pre) malignant lesions were found on the nose, pinnae, dorsa of hands, scalp and the face; a cutaneous horn found in one of these sites was 2·1 times more likely to have derived from a (pre) malignant base than any other parts of the body ($P < 0{\cdot}001$). (d) Geometry of the lesion: lesions with a wide base or a low height-to-base ratio were more likely to be (pre) malignant ($P < 0{\cdot}0005$).

The incidence of squamous cell carcinoma in actinic keratoses giving rise to cutaneous horns was 9·5%. This figure showed no significant difference ($P > 0{\cdot}2$) when compared to the 6·2% found in actinic keratoses without horns during the same period. The presence of a cutaneous horn did not significantly increase the risk of an actinic keratosis progressing into a frank squamous cell carcinoma.

Necrobiosis lipoidica: 'the footprint not the footstep'

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Necrobiosis lipoidica is a chronic, idiopathic focal destructive disease of the dermis. Our aim was to characterize the clinical and histological features in an attempt to understand the nature of these lesions. The diabetic population of Nottingham and Derby is 6305 and the prevalence of necrobiosis in this population is 1·2%. Over 12 months, 35 patients suffering from necrobiosis lipoidica (27 females and 8 males) of whom 31 were diabetic, 25 insulin-dependent and six not, (ages 17–76 years) were recruited. Necrobiosis was diagnosed at a much younger age in the insulin-dependent group (mean 22 years) when compared to the combined non-insulin-dependent and non-diabetic groups (mean 49 years; $P=0{\cdot}01$, Mann–Whitney *U*-test). There was a mean delay of 10 years between diagnosis of diabetes and onset of necrobiosis. Glycaemic control as measured by HbA1 at the initial visit was 11·5% for the necrobiotics which is not dissimilar to the mean (11·3%) for diabetics attending the hospital. The sites affected were the shins, calves, dorsae of feet and upper limbs. Seventy discrete lesions were observed, 30 had ulcerated and 73% showed evidence of reduced local sensation. Ulceration correlated with sensory impairment ($P<0{\cdot}05$ chi-square) and occurred only in diabetics. It was independent of age, lesion size, duration of necrobiosis, peripheral arterial insufficiency or neuropathy. To establish whether necrobiosis targeted specific dermal structures or non-selectively destroyed the dermis, eccrine function from lesional and normal skin was assessed by evaporimetry and iontophoretic sweating. Sweating was significantly reduced over the necrobiotic areas ($P<0{\cdot}01$ Wilcoxon sign rank test). We believe these abnormal physical properties are a consequence of the destruction of dermal structures by the necrobiotic process, and are not related to its pathogenesis. Punch biopsies were obtained from the edge of lesions. Histological sections demonstrated a spectrum of findings from active collagen degeneration with histiocytic reaction to dense scarring with no cellular reaction. To obtain more information about its aetiology and pathogenesis, further examination is required of the inflammatory infiltrate in early lesions or perilesional skin.

Topical mechlorethamine: an effective treatment for skin involvement in Langerhans cell histiocytosis

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Involvement of the skin is common in Langerhans cell histiocytosis (LCH), especially in younger children and particularly in those with multisystem disease. Though skin involvement may be responsible for a variety of unpleasant symptoms, conventional topical treatment has proved to be of very limited benefit. We believe that expectant treatment is indicated in the great majority of cases of LCH, as spontaneous resolution is frequent. However, a conservative approach to treatment of LCH in children requires that effective symptomatic therapy of cutaneous disease should be available. We have found topical mustine to be exceptionally valuable for this purpose. Of 61 children with LCH seen during the last 9 years, 43 had cutaneous involvement, of whom 16 were treated with topical mustine. All of these were demonstrated to have multisystem disease. Response to daily applications of a solution containing mustine HCl 20 mg/100 ml was rapid. All these patients achieved a full cutaneous remission within 7 months (11/16 within 2 months), except two children in whom the treatment formed part of terminal care. In all cases, remission was sustained for at least 6 months. Six required further treatment with equally good response. Adverse reactions were not seen, with the exception of contact sensitization in one child.

Platelet and coagulation studies in Ehlers Danlos syndrome

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Ehlers Danlos syndrome (EDS) is often complicated by a bleeding dyscrasia. Despite being a well-recognized 'textbook feature' of EDS there are few reports of bleeding or bruising in the EDS literature. In Ehlers Danlos Type X, the underlying biochemical defect has been identified as a deficiency in fibronectin, which accounts for both the connective tissue and platelet abnormalities observed in this condition.[1] To determine the incidence and nature of platelet, bleeding and coagulation abnormalities in other types of Ehlers Danlos syndrome, we studied 32 patients in detail. Twelve patients had type I EDS, 11 had type II EDS, seven had type IV EDS and two had type VIII EDS. Haematological investigations included platelet count, bleeding time platelet aggregation studies, Factor XIII assay, coagulation screen and fibrinogen titre.

Fourteen of the patients had no detectable haematological abnormality. Four patients had prolonged bleeding times. All the tested patients had normal platelet counts. Eighteen patients had reduced or absent platelet aggregation to one or more of the aggregometry test reagents (collagen, ADP, arachidonate, ristocetin, adrenaline). Two patients had greatly reduced Factor XIII assays. Two patients had prolonged coagulation times, one with a reduced level of fibrinogen.

We conclude that platelet and coagulation abnormalities in EDS are more common than had previously been realized, and may contribute to the bleeding dyscrasia in this condition.

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Infusion of iloprost in Raynaud's phenomenon: lack of antiplatelet effect but risk involved

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In vitro studies have demonstrated that prostacyclin (PGI_2) is the most powerful antiplatelet/antithrombotic drug. Short-term infusion of a stable analogue of PGI_2 (iloprost [I]) has been recommended for the treatment of Raynaud's phenomenon in patients with severe systemic sclerosis. Intravenous infusion of [I] was given to 10 such patients, according to manufacturer's recommendations, on 3 consecutive days, for 8 h starting at a rate of 0·5 ng/kg/min and increasing by 0·5 ng/kg/min every 15 min to a maximum of 2·0 ng/kg/min. The thrombotic status of patients was assessed by a new technique (haemostatometry) which allows measurement of platelet function such as shear-induced haemostasis and thrombus formation on collagen fibres from a non-anticoagulated venous blood sample. Coagulation of the blood was also monitored. Haemostatometry was performed before the infusion, 4 h after its commencement on the third day and 1 h after completion of the infusion.

There was a complete lack of any antiplatelet effect on haemostasis and thrombus formation on collagen of [I] during and immediately after the infusion. Conversely, platelet hyperreactivity and enhanced coagulation were observed during the infusion. *In vitro*, a potent antiplatelet effect with greatly prolonged coagulation was observed in the 1·6–8·0 ng/ml range of [I], which is 25–125 times higher than that achieved in the blood of patients. Enhanced platelet reactivity and coagulation was observed *in vitro* at the concentration which corresponds to the peak plasma level of [I] in patients. Thus our study, using the recommended dose of [I], failed to show any antiplatelet effect, but demonstrated a risk of thrombotic complication, due to the previously undetected effect of low dose of [I] on platelets and the coagulation system.

Activated peripheral blood T lymphocytes in atopic dermatitis

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T helper/inducer (CD_4) lymphocytes are the main component of the cutaneous infiltrate in atopic dermatitis (AD), with ratios of CD4 to T suppressor/cytotoxic (CD8) cells as high as 7:1.[1] Previous studies have failed to demonstrate a consistent abnormality of peripheral blood lymphocytes in AD. We have investigated the degree of T lymphocyte activation in the blood of patients with AD by measuring the expression of DR antigens by CD4 and CD8.

Six patients with refractory AD (mean age 34 years) were studied over an 8-week period. Patients continued their standard topical treatment and did not receive systemic therapy. Disease activity was measured by a single observer assessing erythema, pus, excoriation/crusting, cracking, dryness/scaling and lichenification on a scale of 0–3 at six defined sites, giving a maximum score of 108. Assessment and venesection were performed every 2 weeks. Control data was obtained from age- and sex-matched normal subjects.

Lymphocytes were harvested by established techniques and stained with monoclonal antibodies (Dako and Becton-Dickinson reagents). Double-labelling techniques allowed the identification of both T-cell subsets and activated T cells. Labelled cells were counted by a fluorescence-activated cell sorter and the absolute lymphocyte count was measured by Coulter analysis.

In patients with AD, the total T, CD4 and CD8 cell numbers and the CD4/CD8 ratio were all within the normal range. Although the numbers of activated (DR+) CD4 cells were elevated ($P < 0{\cdot}05$), levels of activated (DR^+) CD8 cells were normal. Increasing disease activity was associated with an increase in the numbers of DR^+CD4 cells ($P < 0{\cdot}05$) with no change in the levels of DR^+CD8 cells. The DR^+CD4/DR^+CD8 ratio increased in proportion to disease activity ($P < 0{\cdot}05$).

The absolute increase in activated CD4 cells in relation to disease activity suggests a major role for CD4 cells in the pathogenesis of AD.

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The ichthyoses: a re-appraisal based on a study of 100 patients

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Our classification of the inherited ichthyoses is based on three main groups: ichthyosis vulgaris or common type, the more severe forms predominantly limited to the skin and the ichthyosiform syndromes associated with multisystem disease.

Twenty-four of 100 patients with inherited ichthyosis were born as collodion babies. Sixteen went on to develop non-bullous ichthyosiform erythroderma, five lamellar ichthyosis of variable severity, one trichothiodystrophy or IBIDS, one a pustular ichthyosiform erythroderma and one does not have ichthyosis but a residual palmoplantar keratoderma.

Twenty-nine of the 100 had bullous ichthyosiform erythroderma with a spectrum of clinical features ranging from mild flexural hyperkeratosis to severe, debilitating generalized disease.

Thirty-two of the 100 have had etretinate therapy (10 for more than 5 years). No major problems have been encountered apart from three patients with questionably abnormal bone scans.

Long-term cyclosporin A in eczematous disorders

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Double-blind trials have confirmed the effectiveness of oral cyclosporin A (CyA) in treating severe psoriasis. Open trials now suggest low dose CyA may also be effective in other severe skin diseases. In four patients with chronic actinic dermatitis (CAD) unresponsive to light avoidance, sunscreens and topical steroids, and in whom azathioprine, systemic steroids or PUVA phototherapy had been unsuccessful, CyA (4–5 mg/kg/day) was effective in inducing remission. Three patients have been maintained on CyA for over 2 years (2–3 mg/kg/day). In the fourth patient, CyA was discontinued after 6 months because of nephrotoxicity. Ten adult patients (four males, six females) have been treated with CyA (3–5 mg/kg/day) for severe atopic dermatitis (AD), the longest for 3 years. Eight out of 10 patients were judged to have responded well. In two patients treatment was unsuccessful despite CyA doses of up to 6 mg/kg/day; however, in both cases CyA blood levels were consistently low. In two patients treated for 2–3 years an increase in plasma creatinine levels to more than 30% above base line necessitated cessation of therapy. A further three patients have required treatment for hypertension. Low dose oral CyA therefore appears to be effective in treating patients with severe CAD and AD. However, careful monitoring of renal function and BP, and dose adjustment, are required. CyA appears to offer a valuable alternative for therapy-resistant CAD and AD, and double-blind trials are now indicated.

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SUMMARIES OF POSTERS

(1) Pretibial epidermolysis bullosa: a report of three families

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Pretibial epidermolysis bullosa (EB) is a rare variant of dominant dystrophic epidermolysis bullosa (DDEB) with distinct clinicopathological features. We have studied three families with this condition and one further case which was undiagnosed until the eighth decade of life. Clinical details of 25 other affected family members were obtained from the seven cases examined. All the families exhibited an autosomal dominant pattern of inheritance with a male preponderance of 2 : 1. The age of onset varied widely from 5 days to 25 years, but was consistent within families. The mean age for diagnosis of the probands was 42 years.

Two patterns of clinical disease were observed. Cases with early onset disease (< 1 year) exhibited blistering and shearing of the skin in response to trauma of the lower legs during childhood. Cases with late onset disease (> 10 years) exhibited nail dystrophy with pruritic papulo-nodular lesions in the pretibial sites. Involvement of other sites was rare, apart from the presence of albopapuloid lesions in four of the seven cases examined. Subepidermal clefts were commonly seen in biopsies of involved skin even in the absence of blistering. Our data confirms that pretibial EB is a rare variant of DDEB with atypical clinical features which can cause considerable delay in diagnosis.

(2) Desmoplastic melanoma: a rare form of malignant melanoma that is usually mis-diagnosed and mis-managed

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Most desmoplastic malignant melanomas (DMM) arise on the head and neck as a complication of lentigo maligna (LM) in elderly patients. Histologically the tumour can easily be mistaken for a benign scarring process or a neurofibroma with consequent delay in correct diagnosis and adequate surgical treatment. We describe the clinicopathological features of seven patients with DMM to highlight the pitfalls in diagnosis. They were white patients comprising four females and three males (age range 56–85 years at presentation). The patients presented with firm to hard deep-seated nodules or plaques, six tumours arising in the head and neck region and one of the shoulder. Some of the patients had noticed a brownish patch preceding development of the tumour but none showed obvious features of an LM at presentation.

Histologically the dermal tumours were composed mainly of elongated, non-pigmented spindle cells with tapering cell processes set in a loosely textured fibromucous connective tissue.

The spindle cells tended to be arranged in well-defined fascicles present at all levels in the dermis and often extending into the fat. Nuclear atypia and mitotic activity were, as a rule, not conspicuous. Both perineural and intraneural tumour spread was noted in some biopsies. Usually serial sections revealed atypical melanocytic hyperplasia in parts of the overlying epidermis and junctional nests were occasionally seen.

It was unusual to find direct evidence of transformation of LM melanoma to a DMM pattern. The histology of biopsies in this series had been misinterpreted as lupus erythematosus and other benign inflammatory or scarring processes, neurofibroma and fibrosarcoma.

Immunocytochemical labelling for S100 protein and with the anti-melanoma antibodies HMB45 and NK1C3 was usually positive in most of the spindle cells. Immunocytochemistry should help prevent errors in the histological diagnosis of this rare type of melanoma.

(3) Evaluation of a unique new compression bandage in the treatment of venous ulcers

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Adequate compression bandaging to reduce lower leg venous hypertension is the most important aspect of treating ambulatory patients with venous ulceration. Conventional methods of providing compression, such as paste bandages with other outer forms of support, have been used for many years but are associated with a number of problems. An ideal bandage would be one that provides adequate gradient compression as well as a local environment which is conducive to improvement of the skin surrounding the ulcer. For the last year we have evaluated a new adhesive compression bandage with a hydrocolloid lining that meets the above criteria (Granuflex (DuoDerm) Adhesive Compression Bandage, ConvaTec.). Preclinical pressure measurements on 10 volunteers demonstrated that the bandage provided adequate gradient levels of compression, as measured by pressure transducers placed at the ankle, gaiter and calf. In a clinical study comparing the hydrocolloid lined compression bandage with a standard paste and outer support bandage on more than 20 patients we found that the new bandage demonstrated efficacy in the healing of venous ulcers. Since the inner layer is hydrocolloid in nature, exudate leakage from the primary dressing over the ulcer can be absorbed, increasing the interval between any bandage changes due to soiling. One of the most striking findings was the gross clinical improvement in the condition of the skin surrounding the ulcer. To investigate this in more detail silicone skin replicates were made on the skin on the affected leg, before and after treatment, with the new bandage. Positive templates were constructed and examined by scanning electron microscopy. Using this technique it was clearly shown that after 3 days of treatment the condition of the skin on the ulcerated leg had greatly improved. In summary, these studies demonstrate that this new bandage provides adequate compression together with improved healing of the ulcer and the surrounding skin.

(4) The sequelae of chronic cutaneous lupus erythematosus

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Skin involvement in patients with lupus erythematosus (LE) has been classified as acute, subacute or chronic.[1] Chronic cutaneous lupus erythematosus (CCLE), which includes localized or generalized discoid LE (DLE); hypertrophic LE and lupus profundus may be limited to the skin or occur in association with systemic LE (SLE). As part of a long-term prospective study of patients with cutaneous LE, we have examined 85 patients with CCLE to determine the prevalence and character of sequelae.

Scarring alopecia was present in 28% (24/85) of patients and this was often difficult to control with topical treatment. Disfiguring depressed cribriform scars on the nose and cheeks were common but the cosmetic disability was accentuated by epidermal pigmentary disturbance: macular hyperpigmentation was a prominent feature in 25% (21/85) of patients. Location of pigment within the epidermis was confirmed by Woods light examination and histology. Ear lobe and concha involvement was common, sometimes leading to progressive destruction of the lobe. Involvement of the lower eyelids was present in 8% (7/85) and in one of these patients ectropion was a troublesome complication. Atrophie blanche with scarring on the plantar surface of the toes was a striking feature in 3·5% (3/85). The sunken scars produced by lupus profundus were particularly disfiguring and in one of the four sufferers there was gross facial deformity.

Scarring oral mucosal lesions were present in 8% (7/85); chronic nasal mucosal involvement was observed in 9% (8/85) and conjunctival scarring was a feature in 2% (2/85). In three patients the intervention of plastic surgeons had been requested to correct deformity. Interestingly, there was no recurrence of disease within the full thickness skin grafts or flaps employed in two of these patients, both of whom had active disease elsewhere. Cutaneous squamous cell carcinomas developed within hypertrophic lesions in two patients, both of whom had typical discoid lesions elsewhere. An atypical fibroxanthoma presented within the inactive scar on the face of one patient.

We conclude that despite the advent of treatment with corticosteroids, CCLE still causes significant handicap and disfigurement. Patients are often lost to follow-up once a chronic relatively stable condition has been identified, so under-treated relapses are common. Aggressive and regular medical and surgical treatment may be required to prevent or correct sequelae, which at best are a cosmetic disability and at worst may result in tissue destruction, deformity or squamous cell carcinoma.

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(5) Peptidergic neurotransmitter innervation of the eccrine sweat gland and their presence in sweat secretions

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Regulatory peptides are biologically active polypeptides which exert control of bodily functions through their actions as neurotransmitters in peptidergic nerves. In this study the technique of immunocytochemistry was used to determine presence and localization of a range of peptidergic neurotransmitters in relation to the human eccrine sweat gland. Peptidergic nerves containing both vasoactive intestinal peptide (VIP) and peptide histidine isoleucine (PHI) were the neurotransmitters found most frequently innervating the human eccrine gland, occurring in the periglandular nerves in close approximation to the secretory cells.

The presence of binding sites for both VIP and PHI were shown on human eccrine secretory cells by means of autoradiography. These peptides were also identified in exercise-induced sweat secretions by means of both radioimmunoassay and rpHPLC. The findings of this study suggest an important role for peptidergic neurotransmitters in the physiological control of human eccrine sweat production.

(6) Inhibition of human epidermal phospholipase A2 by polyunsaturated fatty acids

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Phospholipase A2 (PLA2) (E.C. 3.1.1.4) is believed to be the rate-limiting enzyme in eicosanoid synthesis. Recent reports have shown that the membrane-bound form of PLA2 involved in arachidonate release is regulated through product inhibition by polyunsaturated fatty acids.[1] In addition, product inhibition of human epidermal PLA2 by arachidonic acid appears to be abnormal in psoriasis. We have investigated the structural requirements for inhibition of human skin PLA2 by polyunsaturated fatty acids. Human epidermal PLA2 was partially purified from epidermis of post-mortem skin by detergent solubilization and ion exchange chromatography. Following SDS/PAGE and Western blotting the enzyme was shown to cross react with antibodies to authentic PLA2, revealing a major band of 68 kDa and a minor band at 16 kDa. The 68 kDa form was converted to the 16 kDa form on storage or dialysis against distilled water indicating that it was a tetramer. Enzyme activity was inhibited by polyunsaturated fatty acids at micromolar concentrations with inhibitory potency increasing with increasing degree of unsaturation (e.g. IC 50% C18:1: > 100 μM; C18:2:85 μM; C18:3:45μM; C18:4:38 μM). For a given degree of unsaturation an increase in chain length decreased inhibitory activity (e.g. IC 50% C18:4:38 μM; C20:4:45 μM; C22:4: > 100 μM). Trans-unsaturated fatty acids did not inhibit enzyme activity (IC 50% trans C18:2 > 300 μM). Similarly, methyl esters and acid chlorides of active free acids were ineffective as enzyme inhibitors. The present results show that human epidermal PLA2 is inhibited by polyunsaturated fatty acids. This may be of relevance in the development of new compounds for the treatment of inflammatory dermatoses such as eczema and psoriasis.

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(7) An immunophenotypic and flow cytometric investigation of adult mastocytosis

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Five adults (aged 40–77 years) with benign mast cell proliferative disease were investigated. All patients had extensive urticaria pigmentosa and two had definite systemic involvement of the bone marrow and spleen; one patient also had hepatic, bone and bowel mucosa lesions. Lesional biopsies and four normal controls were stained by a standard unlabelled antibody bridge APAAP, immuno-alkaline phosphatase using monoclonal antibodies specific for CD1, CD3, CD4, CD7, CD8, CD19, CD22, CD25, CD45, HLA-DR, Dako macrophage, BRC, Dako myeloid/histiocyte, a mast cell granulate specific antibody (Rimmer MCG 35) and IgE Fc receptor (CD23). Flow cytometry (EPICS 'C') was performed on circulating mononuclear cells labelled with monoclonals for CD1–5, CD8, CD19, CD25, HLA-DR, IgM and kappa and lambda light chain. Mastocytosis lesional mast cells differed from controls in expression of DR in four out of five. Mast cells from patients and controls expressed leucocyte common antigen (CD45) and Dako macrophage positivity. CD4 was ± in three patients and + in one, CD23 was ± in four patients and controls and + in one patient. Flow cytometry revealed an increase in peripheral blood T cells expressing activation markers CD25 and HLA-DR. Mast cells from human skin thus bear some markers of macrophage/monocyte lineage suggesting a common precursor in ontogeny (post-pluripotential stem cell).[1] HDL-DR expression by mast cells appears to be a feature of mast cell proliferation which is accompanied by a marked activation of T cells. Such T lymphocytes, possibly via cytokines (?IL-3?IL4) may contribute to the proliferation of the mastocytosis cells.

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(8) H1 antagonism reveals the non-histamine component of antigen weals: a model for further study?

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Antihistamines incompletely suppress urticaria despite their effectiveness on histamine weals. Potent H1 antagonists have less effect on weals due to antigen than those to histamine, but this effect has been shown at a single time point. This study aimed to characterize the part of the antigen weal not due to histamine by comparing the effect of ceterizine on the time course of weal reactions to histamine, codeine (which causes non-immunological mast cell degeneration) and antigen. Ten volunteers sensitive to house dust mite (HDM) received three intradermal injections of 4 μg histamine, 10 μg codeine and 5–20 SQ units HDM before and after ceterizine 10 mg daily for 7 days. Skin thickness was measured with calipers before and at 15 time points after the injections.

Mean maximal weal thickness was 1·37, 1·39 and 1·35 (SE means 0·1) mm for histamine, codeine and HDM. Histamine and codeine weals appeared rapidly, peaked at 10 min and disappeared exponentially. In contrast, HDM weals had a slower appearance, peaked at 20 min, and had a rate of disappearance half that of histamine and codeine. The mean (± SE) maximum inhibition of ceterizine was 93 ± 3% for histamine, 78 ± 6% for codeine and 69 ± 7% for HDM. The time course for HDM after ceterizine revealed a rise in skin thickness maximal at 1–2·5 h which was unaffected by the HI antagonist and quite dissimilar from that seen with histamine and codeine. These observed differences indicate that non-immunological mast cell degranulation results largely in the release of histamine, whereas IgE-mediated mast cell degranulation produces a significant non-HI component, the study of which may lead to development of more effective treatment in urticaria.

(9) Clear UV lenses: cosmetically acceptable spectacles for PUVA patients

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Cataracts are a potential complication of PUVA therapy and patients are therefore required to wear UV blocking spectacles on treatment days. Spectacles currently recommended are usually tinted, often resulting in poor compliance because of poor colour perception, poor definition in conditions of low background light and the fact that some patients find tinted spectacles cosmetically unacceptable. The present study was carried out to assess the suitability of clear 'UV blocking' lenses for use by PUVA patients.

In this study we assessed a number of clear UV coatings and impregnated lenses available in the U.K. Their suitability for PUVA patients was determined by the efficiency with which they blocked out UV radiation below 400 nm, this being assessed using a deuterium discharge lamp and a UV double-grating spectroradiometer. Acceptable transmission was defined as: 390 nm < 10%, 380 nm < 5%, 370 nm < 2% and 360 nm < 1%.

Of the materials tested, Orcolite UV 400 and Orma UVX coatings produced by Norville Optical Limited and Essilor Limited, respectively, were suitable for patient use, and these can be applied easily and cheaply (via opticians) to any plastic lens. Of the impregnated lenses, the clear Sola UV Gard 400 lens and the minimally tinted Polaroid polarizing lenses were also found to be suitable. In the case of the coatings, these cannot be applied to glass, but coated non-prescription or prescription plastic lenses can be purchased at a cost little different to that of Polaroid lenses.

(10) Nodular melanomas in the over-50 age group: the next target for health education

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Pathology records of all patients with melanoma in our district, 1986–89 inclusive, were examined to identify patient and lesion characteristics associated with increasing thickness. A priori we felt that older patients with nodular melanomas (NM) would have thicker lesions. There were 182 patients: 62 with NM, 104 with superficial spreading melanoma (SSM) and 16 with other histogenetic types; 67 male, 115 female; mean age 54 years (SD 17); median Breslow thickness 1·1 mm (range 0·03–10·0). NMs and SSMs were compared. One amelanotic melanoma in a 47-year-old was 10 mm thick and a clear outlier, and this case was excluded. Breslow thickness was missing for four NMs and two SSMs, and age was missing for one NM. The results were as follows: mean age NM:SSM, 56(SE 2·2):52(SE 1·7) years, $P=0·14$; mean thickness NM:SSM, 2·58(SE 0·23):1·12(SE 0·10) mm, $P<0·00005$; M:F ratio, NM 26:33, SSM 33:69, $\chi^2=2·47$, $P>0·05$. NMs showed a much greater correlation between thickness and age ($r=0·44$, $P=0·0005$), than SSM ($r=0·27$, $P=0·0063$). NM was more common in patients >50 (NM:SSM, 42:56) than <50 years old (NM:SSM, 19:48), but the difference was not significant ($\chi^2=3·59$, $P=0·06$). The thickest lesions were NM in patients of >50 years (mean 2·97, SE 0·30), which were nearly twice as thick as those of <50 (mean 1·65, SE 0·22). SSMs of >50 years (mean thickness 1·25, SE 0·15) were thicker than SSMs of <50 (mean 0·97, SE 0·13). Student–Newman–Keuls test showed significant differences between NMs over the age of 50 and all other groups ($P<0·05$), but no differences between other pairs. The results suggest that the proportion of NMs rises with age and is associated with increasing thickness; if confirmed, this would have major implications for health education.

(11) The comparative immunology of the specific dermatoses of pregnancy

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Pemphigoid gestationis and polymorphic eruption of pregnancy are inflammatory gestational dermatoses. Pemphigoid gestationis is a rare bullous condition most frequently seen in multigravid women, whereas polymorphic eruption of pregnancy is more common and usually affects the primigravid. We have analysed the immunological basis for these conditions.

Pemphigoid gestationis results from an allogeneic reaction between maternal lymphocytes and paternal MHC molecules expressed on the placenta. A population of HLA-D positive spindle cells may be detected in both placental villous stroma and focally within the trophoblast. Complement-fixing antibodies are formed against a protein constituent of the placental basement membrane, present also in skin. The antibodies are deposited in both epidermal and amniotic basement membranes and may be pathogenic inducing dermal–epidermal separation and basal cell degeneration. The inflammatory cellular infiltrate is an admixture of eosinophils and neutrophils.

In polymorphic eruption of pregnancy, antibodies against the basement membrane are not produced. The skin lesions comprise spongiotic vesicles with occasional pyknotic keratinocytes with lymphocytic and monocytic microabscesses. Analysis of the cellular infiltrate suggests that this disease is the result of cell-mediated rather than antibody-mediated attack. The placentae in polymorphic eruption of pregnancy are normal and there is no abnormal expression of HLA-D molecules.

(12) Topical nedocromil sodium: a double-blind placebo-controlled study in atopic dermatitis

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Nedocromil sodium is the disodium salt of a pyranoquinoline dicarboxylic acid and has some features in common with sodium cromoglycate. It is effective *in vivo* in the treatment of asthma and allergic conjunctivitis. *In vitro*, it is more active than sodium cromoglycate in preventing the release of inflammatory mediators from mucosal mast cells. However, nedocromil blocks the late-phase cutaneous reactions, but not the weal and flare response, in mast-cell-dependent allergic reactions.[1] As the lesions of chronic atopic eczema show increased numbers of mast cells in the dermis, we investigated the efficacy of nedocromil sodium in the management of this disease.

Thirty-two patients with chronic atopic eczema were enrolled into a randomized double-blind trial of 4% nedocromil sodium cream or matching placebo. Following a 2-week baseline period the drug or placebo was used for 4 weeks. Clinical assessment was made with a graded severity score, area charts using a modification of the rule of nines and patient diary cards recording symptoms of itch, redness and weeping. Total and specific IgE levels were measured before and after the treatment period.

Thirty-one patients completed the trial. No significant differences were found between treatment and control groups as determined by clinical assessment and IgE levels.

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(13) Important plant allergens in the United Kingdom

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Although allergic contact dermatitis has been reported from an increasingly large number of plants, most cases of dermatitis are due to a relatively small number of plant groups in the U.K.

Primula obconica remains the commonest house plant. The *Asteraceae* (daisy family) are increasingly recognized as common causes of weed dermatitis, in particular *Taraxacum officinale* (dandelion). Not all individuals will be detected by screening with sesquiterpene lactone mix. *Alstroemeria* spp. are important allergens in florists and *X Cupressus leylandii* and *Hedera* spp. (ivy) in gardeners. Finger-tip dermatitis is commonly caused by bulbous genera such as *Narcissus*, *Tulipa* and *Hyacinthus*, and *Allium* in food handlers. This poster describes the important botanical features of these plant families.

(14) Homology between glutenin and human connective tissue proteins

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Although gluten is identified as the cause of dermatitis herpetiformis, the exact mode of pathogenesis remains unknown. Immunological cross-reactivity between gluten and skin components is one of the possible mechanisms. To ascertain if molecular mimicry between gluten and human proteins exists, gluten proteins (gliadin and glutenin) were compared to a large protein databank (source P.I.R.) containing 4721 sequences. For each gluten protein assessed, the 20 most homologous amino acid sequences were identified and ranked by computer-assisted analysis.

Proteins found to be homologous with glutenin included human procollagen alpha 1 (III) chain precursor (21·7% identity in 669 amino acid overlap), human collagen alpha 1 (IV) chain (25·7% identity in 569 amino acid overlap), and human collagen alpha 1 (II) chain precursor (20·8% identity in 226 amino acid overlap). Glutenin was also found to have a high degree of homology with a separately taken example of mammalian elastin (23·2% identity in 302 amino acid overlap). Connective tissue proteins were not, however, homologous with gliadin.

There is a close degree of amino acid homology between glutenin and connective tissue proteins. Glutenin has a similar tertiary structure to elastin,[1] which exists as microfibrillar bundles in the papillary dermis. It is not known if this molecular mimicry between glutenin and connective tissue proteins is of pathogenic importance, but this warrants further study.

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(15) Plantar fibromatosis

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Dupuytren, in his original report on palmar fibromatosis, described cases with plantar involvement. Today Dupuytren's contracture is an easily diagnosed condition while its plantar equivalent is poorly recognized and often mis-diagnosed. This poster describes the clinical features of five cases and the characteristic histological findings. The correct diagnosis was not made initially in any of the cases. In three cases hypertrophic scarring or keloid formation was suggested both clinically and histologically. In two patients the extent of involvement led to significant disability and one of them was treated by surgical excision. If surgery is undertaken for this condition it should involve removal of the entire plantar fascia.

(16) The cytokeratin profile of normal vulval epithelium and vulval lichen sclerosus

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An immunohistochemical study was carried out using monoclonal antibodies to cytokeratins, the majority of which were cytokeratin specific. The normal vulva demonstrated interesting regional differences in its cytokeratin profiles. The labia minora, which represent a junctional zone between normal cornified epithelium and a mucosa, had positive staining with the antibodies for 6 and 16. These cytokeratins characterize hyperproliferative epithelia. The cytokeratins 4 and 13 are usually co-expressed in non-cornifying epithelia, and in the labia minora there was expression of cytokeratin 13 with either absent or partial expression of 4. In some cases of lichen sclerosus the cytokeratin profile differed, in that there was some loss of expression of cytokeratin 13 and additional positive staining with the antibody C46, which is a marker for cytokeratins 7 and 17.

The hyperproliferative pattern found in the labia minora may be significant in that the majority of squamous cell carcinomas arising in lichen sclerosus do so on the labia minora.

(17) Contact urticaria due to the common stinging nettle (*Urtica dioica*): histological, ultrastructural and pharmacological studies

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The stinging nettle (*Urtica dioica*) is one of the commonest causes of contact urticaria. The urticaria is accompanied by a stinging sensation lasting longer than 12 h. Little is known of the cellular and molecular mechanism of nettle urticaria. The cutaneous cellular response was examined in six people at 5 min and 12 h after nettle contact. On histological examination of mononuclear, polymorphonuclear and mast cell numbers in nettle-challenged skin, only mast cells were significantly increased at 12 h. Ultrastructurally, some mast-cell degranulation was seen at 5 min and 12 h. At 12 h mast cells were closely associated with dermal dendritic cells suggesting a functional unit. The mean histamine content of a single nettle hair was 6·1 ng. Nettle sting extracts did not release histamine from dispersed rat skin mast cells *in vitro*. These results suggest that part of the immediate reaction to nettle stings is due to histamine. However, the persistence of the stinging sensation might suggest the presence in the nettle sting of substances toxic to nerves or capable of secondary release of other mediators.

(18) Strimmer dermatitis

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The development of powered plant cutters with a rapidly rotating nylon fibre cutting edge (strimmers) has produced a new form of phytophotodermatitis which may be mis-diagnosed because of its unusual morphology and distribution.

We have recently seen three cases and these are described. All three patients developed a maculopapular rash with multiple bright red irregular papules, over the anterior chest wall and arms. Although a few linear lesions were present they were not a prominent feature. The eruption persisted for 2–3 days and then gradually subsided to leave hyperpigmented areas. Direct questioning revealed that each patient had used a strimmer to clear plant undergrowth, on a sunny day, with chest and arms exposed, 12–24 h before the onset of the rash. *Heracleum mantegazzianum* (giant hogweed) was implicated in two cases and *Anthriscus sylvestris* (cow parsley) in the third case. Histological examination of a skin biopsy in one case showed a dermal capillaritis with a pericapillary lymphocytic infiltrate.

The present patients are unusual in that the rash was predominantly maculopapular rather than linear and was widely distributed. Small fragments of plant material and sap are thrown up at considerable velocity by the strimmer and may impinge on exposed skin. Despite an early report[1] the distinctive appearance of strimmer dermatitis is not well known.

Manufacturers of strimmers recommend that protective clothing and goggles should be worn, but compliance may be reduced on sunny days when the potential for phototoxic reactions is at its greatest.

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(19) The use of fluorescent lectin stains to identify fungi in clinical material from skin

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The identification of fungi causing infections of the skin depends on the demonstration of organisms by direct microscopy and by culture, a time-consuming process. With the finding that different lectins have different binding affinities for fungal cell walls, depending on their sugar residue content,[1] a new method of identifying fungi in skin is possible. This study used a panel of different fluorescein conjugated lectins (photohaemagglutinin (PHA), concanavalin A (Con-A), *Ulex europeus* (UE) and *Phytolacca americana* (PLA)). The methods were standardized using whole culture mounts and were then applied to skin scrapings or smears fixed to slides. Discrimination between different fungal pathogens was possible, e.g. *Pityrosporum ovale* labelled with Con-A whereas *Hendersonula* stained best with both Con-A and PLA. Dermatophytes and *Sporothrix schenckii* produced similar reactions, but *Candida albicans* only labelled with Con-A. The principle differences were between yeasts and hyphal fungi, although intensity of staining with Con-A and PLA could be used to distinguish between different mould fungi.

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(20) Mediators of leucocyte infiltration in human skin: a review of recent findings

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Recent research has revealed the presence of a number of novel leucocyte attractants in samples from human skin. A review of our recent findings is presented.

Analysis of normal skin chamber fluid and extracts of heel stratum corneum has confirmed previous reports of the presence of interleukin-1 (IL-1) activity. High performance liquid chromatography (HPLC) purified IL-1a-like material from these sources induces potent, leucocyte-rich inflammatory reactions when re-injected intradermally in the original donors. This material, although present in normal epidermis, appears not to be biologically available, possibly because it is retained intracellularly in keratinocytes in association with membranes, and is shed on the surface in the stratum corneum. Membrane perturbation as may occur in disease or following wounding may allow release of preformed IL-1 and the induction of inflammation.

Normal skin chamber fluid and heel stratum corneum also contain a novel, low molecular weight, non-peptide, lipid-like lymphocyte attractant which is found in normal plasma and which we have termed plasma-associated lymphocyte chemoattractant (PALC). We have proposed that this molecule may induce physiological lymphocyte trafficking in normal skin and possibly other organs.

Analysis of psoriatic lesions stratum corneum extracts by bioassay in combination with HPLC and neutralizing antibodies, has demonstrated the presence of the novel lymphocyte and neutrophil attractant cytokine, IL-8, which was not found in normal skin samples. Analysis of these psoriatic samples for at least eight other cytokines revealed that none other was present in elevated, biologically active amounts relative to extracts of normal stratum corneum.

IL-1, IL-8 and PALC are potentially important mediators of leucocyte infiltration in human skin and may become targets for future anti-inflammatory or immunosuppressive therapy.

(21) The PUVA-associated squamous cell carcinoma: a distinct clinicopathological entity

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Since 1976 there have been over 500 patients treated by photochemotherapy with PUVA for psoriasis at the London Hospital. Their ages have ranged from 16 to 80 years. Ninety per cent of these have been Caucasian, and of this group 15 (3·1%) have developed 72 cutaneous tumours.

The total number of tumours in these patients is made up as follows: 48 squamous cell carcinomata (29 in one patient), four keratoacanthomata, 10 Bowen's disease, nine basal cell carcinomata and one malignant melanoma. The dosage received before tumours developed ranged between 66 and 3804 joules with an average of approximately 1000 joules. Six of the 15 developed tumours after PUVA was discontinued, three of these having previously received Razoxane, shown to be associated with cutaneous malignancies.

The squamous cell carcinomata were recognized clinically as tender, rapidly growing, raised red nodules with a peripheral scale. They usually presented 2–3 weeks after development, having reached by this time a diameter of approximately 1 cm. The histopathology was that of a well differentiated, but invasive, squamous carcinoma. They characteristically did not metastasize, but recurred locally in one of our patients after apparently complete excision. The tumours were studied with a panel of monoclonal antibodies which have shown a loss of markers of differentiation.

The PUVA-associated squamous cell carcinoma is a distinct clinico-pathological phenotype.[1] Continued development of this and other cutaneous tumours after PUVA has been discontinued makes long-term follow-up of patients mandatory.

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(22) Cultured keratinocyte allografts in dystrophic epidermolysis bullosa: preliminary observations

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Cultured allogenic keratinocytes (KC) are used in the treatment of chronic venous ulceration and burns, acting as a potent stimulus to wound healing either via soluble factors or by providing a biological dressing. Patients with epidermolysis bullosa (EB) require repeated skin grafting and plastic surgical procedures. We are investigating the effect of KC on healing time, patient acceptability and the quality of wound repair in patients with dystrophic EB. KC were applied to the donor sites in six patients (five female, one male; aged 15–48 years) with recessive dystrophic EB undergoing various plastic surgical procedures involving split skin autografts. Healing was assessed by weekly photographic clinical records, daily pain charts and biopsies taken from the KC grafted areas and non-grafted sites, as controls, at 7–15 days. Indirect immunofluorescence (IIF) studies using anti-laminin and anti-types IV and VII collagen antibodies were performed and graded 0 to + + +. Clinically, four out of six KC-treated areas healed more quickly than controls by 7 days. Pain was infrequently reported in all patients; three denied any pain. Biopsies, up to day 10, in two cases (Patients 1 and 2) showed flat, well-differentiated epidermis in the KC-grafted areas and controls; a third case (Patient 3) had no epidermis in either area at day 10 but by day 15 it was apparent in both sites. In the remaining cases, epidermis was absent either in the KC-treated or control areas. In Patients 1 and 2, IIF revealed a more intense expression of the basement membrane (BM) components in the allografted sites compared with non-grafted controls, but this was not confirmed in Patient 3. There was no difference in anti-type VII collagen staining between KC-grafted samples and pre-graft skin.

These preliminary findings show that KC allografts may alleviate post-operative pain in EB patients and promote healing, possibly through enhanced expression of certain BM components. However, their effect on this and other aspects of healing requires further study.

(23) Rosacea: a survey of presentation patterns and response to therapy

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Sixty-two consecutive out-patients with rosacea were studied. The sex ratio was equal. The distribution of age at presentation was bimodal with the peak incidences in the 4th and 7th decades. There was a female proponderance (1·2:1·0) in the over-50 age group although four of the five under 30 were men. The commonest symptom was erythema (present in 61 out of 62), followed by papules and pustules (found in 50 and 44, respectively) and telangiectasia and flushing (31 each). Lymphoedematous rosacea was unexpectedly common, being seen in 15, two-thirds of whom were men. Rhinophyma, present in 11 men and one woman, was noted exclusively in the older age group. The cheeks, forehead and nose were common sites but five had peripheral lesions (on the limbs or trunk). The sun or heat were provoking factors in 15 cases, as were topical steroids in 12. Seborrhoeic dermatitis co-existed in four subjects, three also had acne and one had discoid lupus erythematosus as well as rosacea. Oral tetracyclines were the first-line treatment in two-thirds and were effective in nine out of 10 cases. Others received erythromycin, co-trimoxazole or metronidazole. Of the seven treated with topical clindamycin, five improved. Twelve of the 15 with lymphoedematous rosacea showed some resolution with tetracycline therapy.

Unlike previous studies we found an equal sex ratio and a bimodal distribution for the age of onset. We also demonstrated an unexpectedly high prevalence of patients with the lymphoedematous variant (some of whom responded to conventional therapy) and showed that topical clindamycin was effective in certain patients.

(24) A case of human catpox/cowpox infection

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At present the domestic cat is the most commonly reported animal to be infected with cowpox virus (CPV) infection in the U.K. However, the primary host for CPV is not the cat or cow but is thought to be a small mammal, most active in the late summer and early autumn, a time when the majority of CPV infections in cats and cows occur. Sporadic cases of human CPV infection have been reported since 1985, usually following contact with an infected animal.

We report a case of human poxvirus infection which occurred in August 1989 in a 19-year-old veterinary nurse at the site of a scratch inflicted by a domestic tom cat with pneumonia. The nurse worked mainly with small domestic animals but had no known contact with CPV. She did not handle sheep, had never been vaccinated against smallpox and had no history of poxvirus infection. The presence of CPV-specific antibodies in her serum was detected by immunoblotting. The cat, a frequent hunter of small rodents and birds, was considered the most likely source of infection even though he had been healthy previously and had no recent skin lesions. A second possible source of the infection was blood and saliva from a deer wounded in a road traffic accident which had contaminated the patient's scratched hand prior to the development of her symptoms. However, cowpox has not been reported in deer, and deerpox is not found in Europe. In addition, no antibodies were detected in the patient's serum which reacted with orf virus, to which deerpox is closely related.

Even though this is only the seventh case reported in the world literature it underlines the potential importance of CPV as a zoonosis contracted from a popular domestic pet, particularly as the incidence of CPV in cats is rising and human vaccination against smallpox, which may be protective, has ceased.

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CASE REPORTS

(1) Eczema, thrombocytopenia and recurrent infection: Wiskott–Aldrich syndrome?

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B.W., male, aged 10 years.

History. This patient has suffered from severe eczema and asthma (associated with upper respiratory tract infections) since the age of 1 month. During the neonatal period he was noted to have an episode of rectal bleeding, but had no further bleeding problems until the age of 4 years when he presented with rectal and nose bleeds associated with easy bruising. No cause was found, other than low platelet counts of about 50,000/mm^3.

Recurrent mild infections have also been a problem, including secondary infection of his eczema, furunculosis, conjunctivitis, pneumonia, varicella, mumps and warts.

Family history. Mother had eczema and hay fever. Maternal grandmother, grandfather and great grandfather were all atopic. Paternal uncle had asthma. No family history of purpura.

Investigations. Platelets $50 \times 10^9/l$ with normal morphology. Bone marrow in 1983 showed increased megakaryocytes but was said to be normal in 1985. He is of blood group A, but anti-B isohaemagglutinins are low at a titre of 1 : 1. T-cell estimations have shown normal CD4 but low CD8 numbers, giving a slightly high 4/8 ration of 2·83. B-cell numbers were low at $0{\cdot}02 \times 10^9/l$ (NR $0{\cdot}1$–$0{\cdot}8 \times 10^9/l$). PHA and PWM responses were normal, but there was a low ConA response consistent with the low CD8 numbers. Immunoglobulin levels were normal except for a raised IgE of 370 units/ml. No antiplatelet or antimegakaryocyte antibodies were found and neutrophil numbers and function were normal.

Patients with the Wiskott–Aldrich syndrome have been shown to lack a 115 kDa glycoprotein on the surface of their lymphocytes.[1] Investigation for this protein was carried out by radio-iodination of the surface proteins of the patient's lymphocytes using lactoperoxidase (which labels tyrosine residues). The cell membrane proteins were then extracted and run on SDS-polyacrylamide gels, the presence of different molecular weight proteins being assessed by autoradiography. Using this technique, the 115 kDa protein was absent.

Diagnosis. It has been unclear as to whether this child suffers from atopic eczema and idiopathic thrombocytopenic purpura, or whether he has the Wiskott–Aldrich syndrome. The lack of the 115 kDa protein, however, confirms the latter diagnosis.

Management. The child's eczema has always posed a therapeutic problem, responding poorly to topical and oral steroids. His eczema has, however, responded well to Synacthen® injections (which have not been associated with problems of infection or purpura at injection sites). Of late, however, the doses required to control his eczema have resulted in growth suppression. After much discussion, therefore, he was started on azathioprine, even though it theoretically could increase his chances of developing a lymphoma in later life (Wiskott–Aldrich patients already having a raised risk of lymphoid tumours of about 13%[2]). There has been an increase in growth following this and much easier control of the eczema.

Bone marrow transplantation is said to correct the haematological immune defects in patients.[3] However, this patient does not have an HLA compatible sibling and would therefore require a matched unrelated graft.

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(2) Congenital systemic mastocytosis

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P.H., male, aged 2 years.

History. This boy was noted by his mother to have abnormally thickened, yellowish, doughy skin from birth. Very early in infancy it was seen that bouts of scratching lead to weals and sometimes blisters. At 2 months of age a clinical diagnosis of diffuse cutaneous mastocytosis was made.

An antenatal ultrasound scan had shown bilateral dilated renal pelvices and post-natally an intravenous urogram had demonstrated bilateral duplex ureters with a right upper hydronephrosis. Prior to elective excision of the non-functioning upper pole of the right kidney at 8 months of age, pre-operative physical examination showed no abnormalities apart from the skin changes. At that stage the haemoglobin was 11·9 g/dl but the total white count was elevated at $16{\cdot}3 \times 10^9$/l with a lymphocytosis of $12{\cdot}7 \times 10^9$/l; platelet count was normal at 427×10^9/l.

As the first birthday approached, it was clear that he was failing to thrive, with a fall in the progression of both height and weight to below the third centile. This was accompanied by paroxysmal diarrhoea. At this stage there was wasting of the buttocks, a distended abdomen and a 2-cm palpable liver edge. A full blood count again showed a leucocytosis of $22{\cdot}3 \times 10^9$/l with elevation of neutrophils ($8{\cdot}9 \times 10^9$/l), lymphocytes ($10{\cdot}3 \times 10^9$/l) and eosinophils ($0{\cdot}5 \times 10^9$/l). A full evaluation was carried out at that time and the only other abnormality was in the jejunal biopsy.

By 16 months of age a number of indurated, erythematous, dermal/subcutaneous plaques had developed on the thighs, over the knees and on the dorsal surfaces of the hands and feet (Fig. 1). Hepatosplenomegaly was now evident with a firm 4-cm spleen palpable. Other investigations at this stage included a normal skeletal survey. The leucocytosis persisted ($21{\cdot}7 \times 10^9$/l).

He was admitted with a view to obtaining a bone marrow aspirate and trephine. Just prior to these procedures he had an acute episode of periorbital oedema accompanied by hypertension (210/140) and a tachycardia of 130/min. There had been six previous episodes of periorbital swelling at home. Blood taken during the episode in hospital showed marked elevation of renin

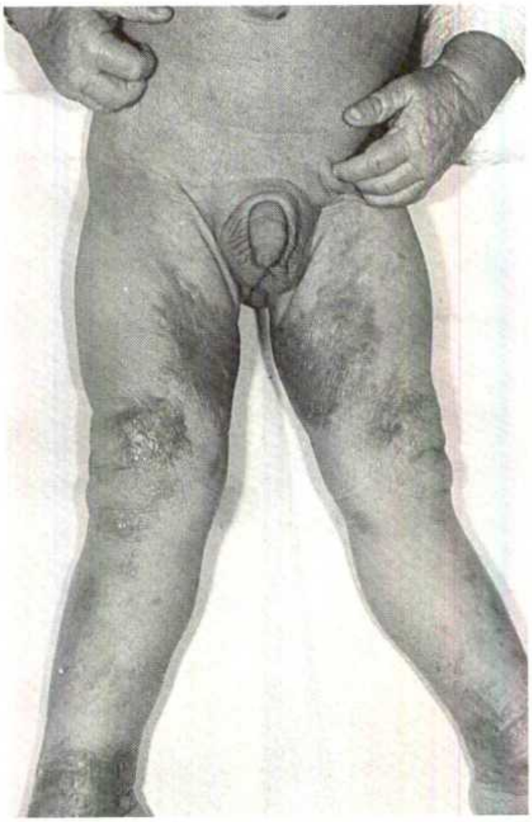

FIGURE 1. Diffuse thickening of the skin with indurated erythematous plaques on the thighs, backs of hands and ankles.

at 44·0 ng/ml/h (normal less than 2·0) and aldosterone at 3313 pmol/l (normal 70–350 resting, 150–750 ambulatory). IV chlorpheniramine was administered and the attack settled overnight without further intervention. Eight hours post-operatively he became agitated, developed a pyrexia of 39°C and developed an acute episode of generalized wealing and haemorrhagic blister formation (Fig. 2). This was not accompanied by any change in blood pressure. There was oozing from the site of a skin biopsy. Clotting studies were abnormal with prolongation for the thrombin time at 19 s (normal 12–15 s), prothrombin time at 21 s (normal 13–17 s) and activated partial thromboplastin time at 65 s (normal 28–40 s). The white cell count was grossly elevated at $56{\cdot}7 \times 10^9$/l with a predominant neutrophilia of $36{\cdot}2 \times 10^9$/l and the platelet count was elevated at 552×10^9/l. The bleeding was controlled with fresh frozen plasma and vitamin K. The clotting tests returned to normal and the blistering settled over the next 4 days.

Since then there has been gradual progression of the indurated plaques and continued pruritus with weals and episodes of haemorrhagic bullae.

Investigations

Skin biopsy. The sample taken at the time of partial nephrectomy showed a diffuse dermal infiltrate of morphologically normal mast cells (Fig. 3); the biopsy taken of an indurated plaque

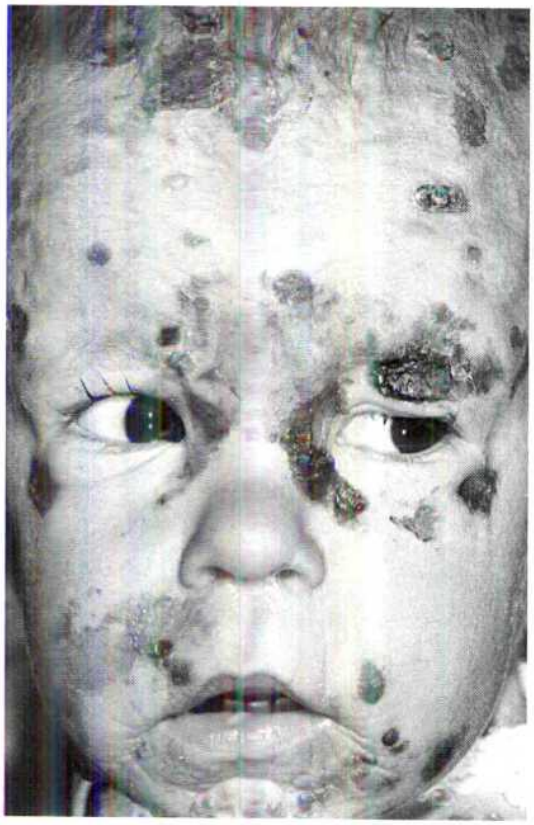

FIGURE 2. Haemorrhagic bullae.

showed mast cells throughout the dermis and fibrosis in the deeper dermis and upper subcutaneous fat (Fig. 4).

Partial nephrectomy tissue. This showed excess numbers of mast cells around blood vessels.

Jejunal biopsy. Normal villous architecture with a dense mast cell infiltrate in the lamina propria.

Bone marrow. There was markedly increased cellularity with very active myelopoiesis. Toluidine blue staining showed increased mast cell numbers.

Cytogenetic studies of peripheral blood lymphocytes and bone marrow cells. No abnormality.

Treatment. Terfenadine, ketotifen and oral disodium cromoglycate have shown little effect. A trial of treatment with aspirin under hospital supervision was terminated because of increased skin blistering and bleeding. He has had a trial course of lymphoblastoid interferon-alpha (Wellcome) with benefit.

Comment. The rarity of systemic mastocytosis in early childhood makes prognostication very difficult. Of the eight previously described cases with an onset in infancy,[1] six occurred in boys and two in girls. Four were associated with a fatal outcome with death occurring at 1, 5, 8 and 27 years. In the other four cases, follow-up to the age of 5 months, 2 years, 2 years and 5 years was presented and no further knowledge of these cases is available. In the four fatal cases death was

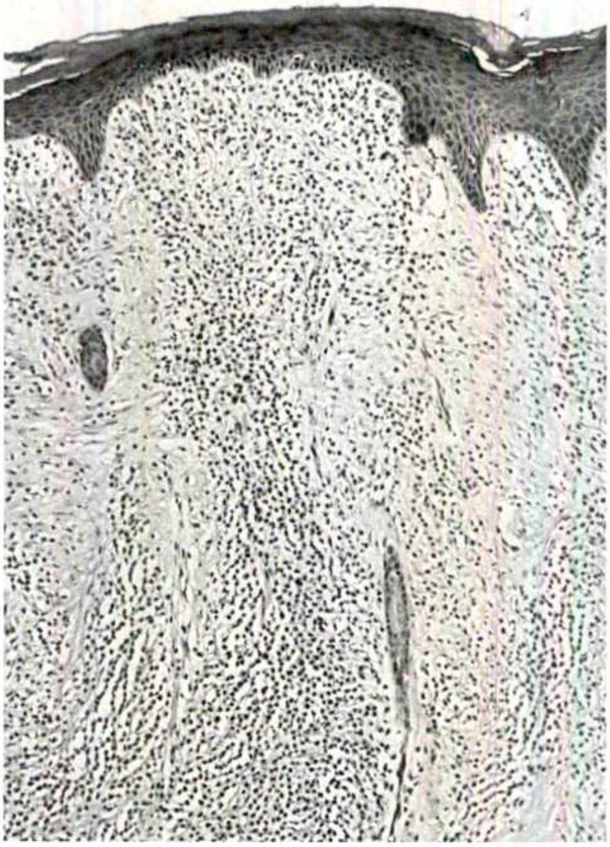

FIGURE 3. Diffuse mast cell infiltration of the dermis.

attributed to acute leukaemia, haemorrhage from a subcapsular liver haematoma, pulmonary oedema and a perforated jejunal ulcer with coexisting myelofibrosis.

Adult onset systemic mastocytosis is relatively much more common and extensive reviews are available.[1,2] In the description by Sagher and Evan-Paz[1] 21 of 71 cases of proven systemic mastocytosis had a fatal outcome, and 80% of these deaths were due to a haematological malignancy (including acute and chronic myeloid leukaemias, myeloproliferative disorders, non-Hodgkin's lymphomas and mast cell leukaemia). A leucocytosis was found to be a poor prognostic feature in 10 of 13 cases, with a raised white cell count greater than 20×10^9 having a fatal outcome. In retrospective analyses of cases presenting to the Mayo Clinic[2,3] the 5-year survival of cases of systemic mastocytosis associated with a haematological disorder (28%) was found to be significantly worse than in those cases of systemic mastocytosis alone.

Many of the symptoms of mastocytosis, particularly those of the acute attacks, are related to degranulation and release of stimulatory substances. These include histamine, heparins, proteases, leukotrienes and prostaglandins. PGD2 has been associated with diarrhoea and PGE2 is known to stimulate renin release. It is possible that mast cells in the region of the juxta-glomerular cells of the kidney could have been responsible for the episode of acute elevation of blood pressure and renin.

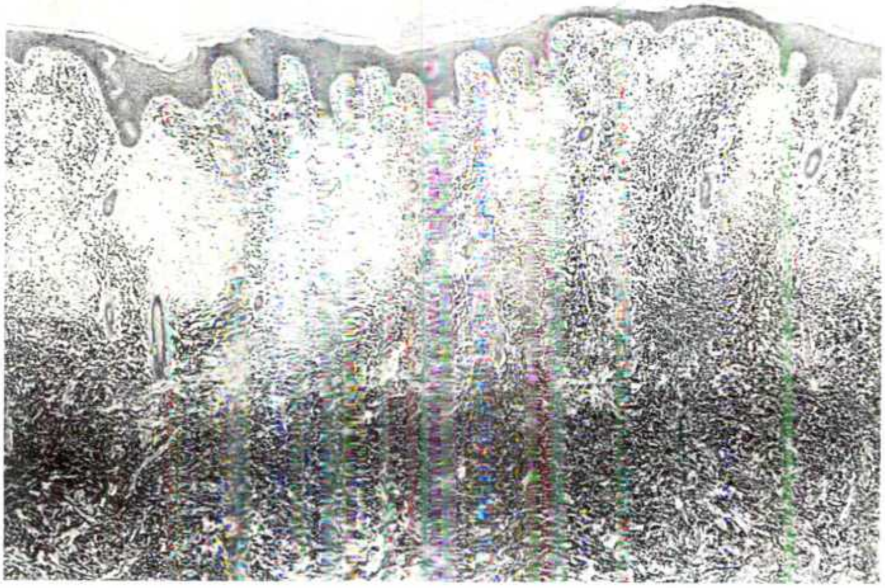

FIGURE 4. Mast cell infiltration and fibrosis of the lower reticular dermis.

Mast cells have been shown to secrete haemopoietic growth factors, and this probably provides the link between systemic mastocytosis and haematological malignancies. Studies are under way in this case to evaluate granulocyte macrophage colony-stimulating-factor (GM-CSF) and other cytokines. Mast cells have also been shown to stimulate fibroblast proliferation and synthesis of collagen precursor proteins.[4]

Conventional therapy for systemic mastocytosis (antihistamines, so-called mast cell membrane stabilizers and prostaglandin synthetase inhibitors) has proved disappointing in this case. Alpha and beta interferons have been shown to inhibit IgE-dependent histamine release from rat mast cells,[5] and alpha interferons have been used widely with some success in controlling myeloproliferative disorders and chronic myeloid leukaemia.

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(3) Familial chronic mucocutaneous candidosis with autoimmune polyendocrinopathy, alopecia and resistance to antifungal therapy

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S.H., female, aged 35 years.

History. No abnormalities were recorded at birth, but keratitis and corneal ulceration occurred at 6 months. Oral, periungual and axillary candidosis were apparent by age 5 years, attacks of jaundice progressing to cirrhosis at age 6, hypoparathyroidism presented at age 7 and Addison's disease at age 9. She had patches of alopecia areata at age 13 which subsequently regrew. At laparotomy for investigation of primary amenorrhoea she was shown to have streak ovaries.

The candidal infection has remained largely confined to the oral mucosa (Fig. 1), nails and nail folds. In 1983–84 she developed dysphagia which necessitated oesophageal dilatation. From 1986 there has been progressive reduction in hair density on the crown of the scalp.

Family history. The parents are not consanguinous. Her mother had alopecia areata. Her brother has chronic mucocutaneous candidosis, chronic keratitis, liver disease, hypoparathy-

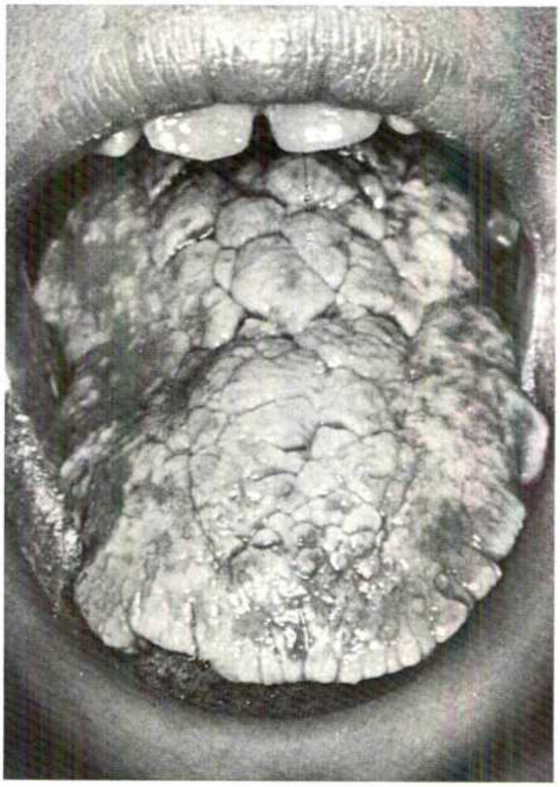

FIGURE 1. Candidosis of the tongue.

roidism, Addison's disease and briefly had a patch of alopecia areata. Her other sibling is unaffected.

Investigations

Mycology. From early childhood *Candida albicans* and occasionally *C. parapsilosis* have been grown from affected mouth, nailplates and skin. In 1979, while on ketoconazole, an isolate was obtained with a slightly elevated minimum inhibitory concentration (MIC) to the drug, and at this time she had low blood levels. Subsequently, the oral *C. albicans* has been resistant to ketoconazole and itraconazole.

Immunology. 1969 (Edinburgh; Dr Irvine). Positive autoantibodies to adrenal cortex, testis and ovary; other autoantibodies negative. 1981 (St. Mary's, London; Dr Valdimarsson). Low B cells, high suppressor/cytotoxic T cells, abnormal PHA response, low Con-A response, absent lymphocyte transformation to Candida, borderline low antibody-independent opsonins, normal neutrophil Candida killing. 1985 (Bristol; Dr Wallington). Low B cells, low suppressor/cytotoxic T cells, abnormal PHA response, borderline low Con-A response, absent lymphocyte transformation to Candida, normal antibody-independent opsonins, no response to intradermal Candida antigen at 48 h. 1987 (Bristol; Dr Wallington). On inosine pranobex: low B cells, normal suppressor/cytotoxic T cells, normal PHA and Con-A response, a reduced lymphocyte transformation to Candida and normal antibody-independent opsonins. A 2-mm weal to intradermal Candida antigen at 48 h.

Iron status. Very low serum iron, low ferritin, high TIBC but only mild hypochromia and microcytosis on blood film and rarely anaemic. Iron values not improved with course of oral supplements. Total dose infusion of iron produced marked elevation of serum ferritin but only a slight trend towards normal in serum iron and TIBC, suggestive of a defect in iron transport.

Management. She has had standard supplements for the hypoparathyroidism, Addison's disease and ovarian insufficiency. The candidosis responded initially to ketoconazole but since resistance developed, only i.v. amphotericin B has been effective. This was combined with thymostimulin TP-1 (Serono), but relapse still occurred. Other attempts at immunomodulation with inosine pranobex and fresh plasma have had no clinical benefit although some of the immunological abnormalities have been reversed. A total dose iron infusion also had no effect on the candidosis.

Comment. This patient and her brother clearly have the autosomal recessively inherited Candida endocrinopathy syndrome; both in addition have had inflammatory eye and liver disease, alopecia areata and iron deficiency. The relevant immunological deficit is not clear from investigations so far. It is an unusual feature of this patient and her brother that both have autoantibodies only to steroid-producing tissues.

Long-term anti-Candidal treatment predisposes to acquired resistance[1] and it is very likely that a period of sub-optimal blood levels of ketoconazole in 1980 contributed to the emergence of the resistant strain the patient still has now.

The alopecia present for the past 4 years has not been fully explained. Diffuse alopecia areata cannot be excluded, since a scalp biopsy has been refused. It is possible, although not proven, that there is metabolism of her steroid hormone supplements to androgenically active metabolites.

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(4) Keratitis ichthyosis deafness (KID) syndrome

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R.W., female, aged 21 years.

History. She was born by forceps delivery at term weighing 8 lb 9 oz. There had been a threatened miscarriage at 8 weeks and the mother had suffered a urinary tract infection at 20 weeks. Membranes ruptured 3 days before delivery and there was persistent pyrexia and diarrhoea during the first 2 weeks after birth.

Thick, rough, non-scaly skin was noted at birth. By 3 weeks of age an erythematous rash had developed in the napkin area and red, crusted lesions developed over the next few months particularly at the angles of the mouth and eyes, on the scalp, in the axillae and around dystrophic finger nails. During infancy a granulomatous reaction to physical injury was noted. Areas of fine follicular hyperkeratosis were noted at the age of 2, particularly involving the inner thighs, groins and umbilicus.

From infancy there have been localized areas of massive hyperkeratosis with chronic infection, usually staphylococcal but occasionally streptococcal (Gram-negative) and candidal.

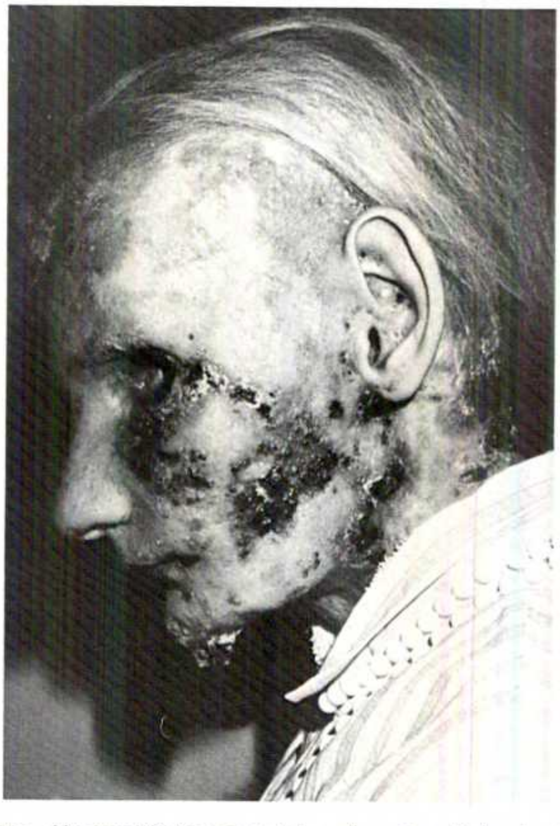

FIGURE 1. Abnormal facies, scanty hair and purulent ichthyotic plaques.

In recent years these have mainly affected the scalp and the feet with severe fissuring and crusting, with scarred plaques elsewhere on the limbs. Since puberty there has been a gradual increase in the extent of the background, flatter uninfected ichthyotic skin.

During early infancy she was admitted repeatedly with pyrexia, otitis externa and skin infections. She also suffered from occasional urinary tract infections and an episode of pneumonia at the age of 7 months, but has not suffered from serious or recurrent systemic infections.

Severe deafness was first noted at the age of 8 months and transtympanic electrocochleography confirmed bilateral neurosensory deafness with no action potential up to 90 decibels.

During early infancy there was recurrent conjunctivitis and blepharitis and ingrowing eyelashes were noted. A grey, lightly vascularized corneal plaque was first noted at the age of 11 months and since then there has been a marked progressive vascular keratitis. Several corneal grafts have been unsuccessful. Evisceration of the left eye was carried out because of recurrent infection and sight is extremely poor in the remaining right eye.

The tongue was noted to be raw at the age of 1 year. White, raised plaques subsequently developed in the mouth and there has been recurrent mouth ulceration.

Family history. No siblings. Parents are not consanguinous.

Examination. Physical examination revealed abnormal facies with a beak nose and deep set eyes, fine pale scalp hair and scanty eyebrows (Fig. 1). Evisceration of the left eye had been carried out and there was a right corneal opacity (Fig. 2). There was fissured angular stomatitis, ulcers along the sides of the tongue and white hyperkeratotic plaques on the buccal mucosa.

There was a widespread, velvety ichthyosis maximal on the trunk with heaped up, crusted and purulent plaques on feet (Fig. 3) and scalp with healed scarred plaques at other sites. There was fine reticulate hyperkeratosis on the volar surface of the fingers and palms obliterating dermatoglyphic markings. The digits were spoon-shaped with dystrophic koilonychial nails.

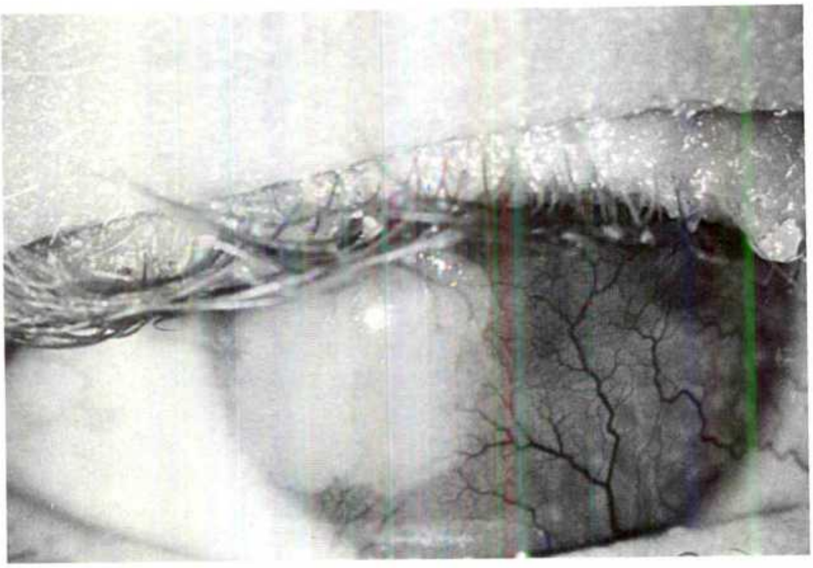

FIGURE 2. Right corneal opacity and vascularizing keratitis.

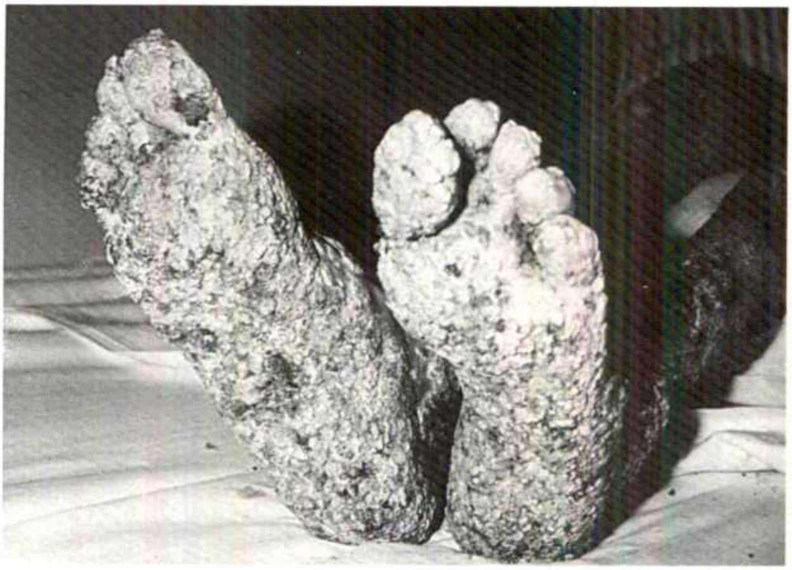

FIGURE 3. Heaped up and crusted skin on soles of feet.

Investigations

Immunology. The following investigations have been normal or near normal on repeated occasions: serum immunoglobulins, lymphocyte subsets, lymphocyte response to phytohaemagglutinin, pokeweed and Candida; nitroblue tetrazolium test with normal polymorphonuclear (PMN) Candida killing and normal PMN chemotaxis; serum complement profile. Both complement functional pathways were active. Antibody-independent opsonins were decreased during active infection but normal following treatment of infection.

Recent results have shown a relatively high lymphocyte count of $3{\cdot}69 \times 10^9$/l (NR 0·95–3·45) with a comparatively high level of helper T cells (CD4) of $2{\cdot}11 \times 10^9$/l (NR 0·45–1·59). The IgG level was considerably raised at 50·96 (g/l); immunofixation was performed and showed no evidence of monoclonality.

We tested the hypothesis that factors at the skin surface were inhibiting opsonization but evidence for this was lacking.

Plasma and urine amino acid patterns have been repeatedly normal. Serum vitamin A level was unrecordable in 1970. Serum zinc level was 5·2 μM/l (normal range 11–13) in 1983. These have both subsequently been normal on several occasions.

Histology. Sections showed marked hyperkeratosis, small foci of parakeratosis, acanthosis with irregular papillary ridges and a fairly prominent granular layer. Dilated sweat ducts and abnormal hair follicles were seen.

Keratin biochemistry. (Dr Skerrow, Glasgow). Revealed immature keratin proteins usually associated with a high turnover epidermis. The tritiated thymidine auto-radiographic labelling index (Professor Marks, Cardiff) was, however, normal (3·2%) indicating a normal rate of epidermal cell turnover.

Microbiology. Culture of skin swabs revealed predominantly *Staphylococcus aureus* (MRSA) occasionally Gram-negative bacteria and *Streptococcus pyogenes*.

Mycology. Occasionally *Candida albicans* has been isolated from skin swabs, and a nail biopsy. Skin scrapings have grown *Trichophyton rubrum* and *Trichophyton mentagraphytes* on rare occasions.

Recently she has developed an iron deficiency anaemia with haemoglobin 8·1 g/dl.

Management. Antiseptics and topical mupirocin ointment have been used regularly with intermittent systemic antibiotics for severe infections. In 1970 vitamin A was prescribed for 3 months. In 1981 she received etretinate 20–30 mg daily which resulted in an itchy, spreading erythematous eruption. She has also received isotretinoin, etretin and arotinoid with similar side effects. Azathioprine 100 mg daily for 3 months produced no improvement. Systemic and topical antifungal agents have produced little benefit.

Comment. This patient was first presented to the B.A.D. in 1973 as 'Dyskeratosis, for diagnosis'.[1] The term keratitis, ichthyosis and deafness (KID) syndrome was first coined by Skinner in 1981[2] and the patient presented here shows the typical clinical features. Cutaneous infection has proved a dominant feature, though extensive investigations have demonstrated a normal immune system. The recent development of a high level of IgE and a high level of helper T cells indicate a stimulatory effect from chronic skin sepsis. Approximately half of the patients described show an increased susceptibility to cutaneous infections. No consistent immunodeficiency has been identified but high levels of IgE, defects in chemotaxis and absence of lymphocyte stimulation by Candida have been reported.[3] However, systemic infections are not a feature and local factors may be responsible.

There are now several documented cases of vertical transmission and the pattern of inheritance suggests an autosomal dominant form of inheritance in these cases.[4] A further complication is the development of squamous cell carcinoma.[4]

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(5) Goldenhar syndrome with unilateral trigeminal trophic syndrome

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D.P., male, aged 3 years.

History. He was born at 35 weeks gestation by normal delivery, the second child of non-consanguinous parents. At birth the following were noted: micrognathia, macrostomia, a nodule on the right cheek (an accessory auricle), a cleft right auricle and a pit on the left cheek. The left ear canal was narrowed. There were feeding and breathing difficulties early in infancy.

From 10 to 11 months there has been left corneal ulceration, and he was first noted to be

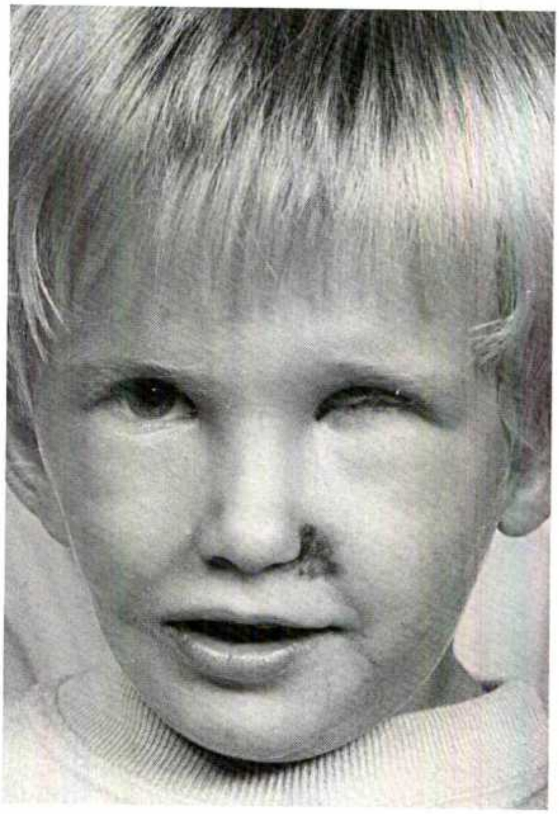

FIGURE 1. Crusting of left nasolabial fold, and a left tarsorrhaphy.

picking the left side of the nose at 1·5 years. Since plastic surgical intervention, physical development has been normal, and there is no indication of intellectual impairment.

There is a normal brother aged 4 years and neither parent shows any morphogenetic abnormality.

Examination. He has a narrow face with a receding chin and associated dental malocclusion. The right external auditory meatus is enlarged downwards and forwards, and is slightly misshapen. There is erosion and crusting in the left naso-labial fold and a left tarsorrhaphy (Fig. 1). He has reduced sensibility to light touch on the left side of the face.

Investigations

Radiology. There is hypoplasia and asymmetry of the mandible, maxillary bones and left petrous temporal bone. The lower cervical and thoracic spine show segmentation anomalies (Fig. 2).

Brain stem-evoked response audiometry. Slightly reduced responses from the right ear, and more severe loss in the left ear (70 dB ± 10 dB loss).

Blink responses. These were assessed while stimulating the supra-orbital nerve and showed a normal response on the right side but no clear response on the left side. There was a probable

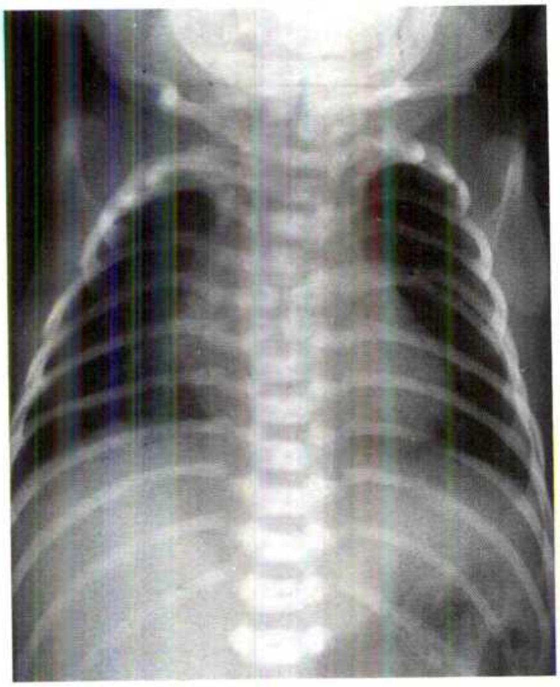

FIGURE 2. Chest X-ray shows 'butterfly' T5 and T6 vertebrae with associated fusion of the posterior ends of the ribs on the left side.

response from contralateral stimulation suggesting a peripheral nerve rather than brain stem defect.

Histology of nodule from right cheek. Consistent with accessory auricle.

Chromosomal analysis. Normal.

Management. The macrostomia was closed, and the nodule on the right cheek excised. A tarsorrhaphy was performed in October 1988 to protect the left cornea. EMLA cream (Astra) and splinting of the arms have not proved helpful for the ulceration of the left ala nasae.

Comment. Goldenhar described a syndrome in which epibulbar dermoids were associated with auricular abnormalities and facial hypoplasia.[1] Since then many other features have been described, and there is some dispute as to which are essential criteria for the condition.[2] There is overlap with oculo-auriculo-vertebral dysplasia[3] and hemifacial microsomia.[4] Nearly all cases of Goldenhar syndrome are sporadic, although a few families demonstrating dominant inheritance have been described.

In Goldenhar syndrome there is hypoplasia of the facial skeleton and musculature. Accessory auricles and pits are common and abnormalities of the axial skeleton are probably a constant feature. Deafness is sufficiently common that it should be tested for at an early age. In some cases there is a cardiac anomaly. Corneal ulceration and the trigeminal trophic syndrome with evidence of sensory impairment do not appear to have been recorded previously.

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(6) Eruptive cutaneous and visceral neonatal haemangiomatosis

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P.B., female, aged 7 months.

History. A few tiny red haemangiomas were noted on the skin at birth. In the second week of life there was a rapid increase in the number of new lesions, all over the skin and in the mouth. A perianal haemangioma eroded and bled, but otherwise the skin lesions have been asymptomatic. Hepatomegaly was noted at a routine check-up at about 4 weeks of age. There have been no features of heart failure, gastrointestinal bleeding and no bruising.

Examination. There were numerous, rounded, red or purple cutaneous haemangiomata, each

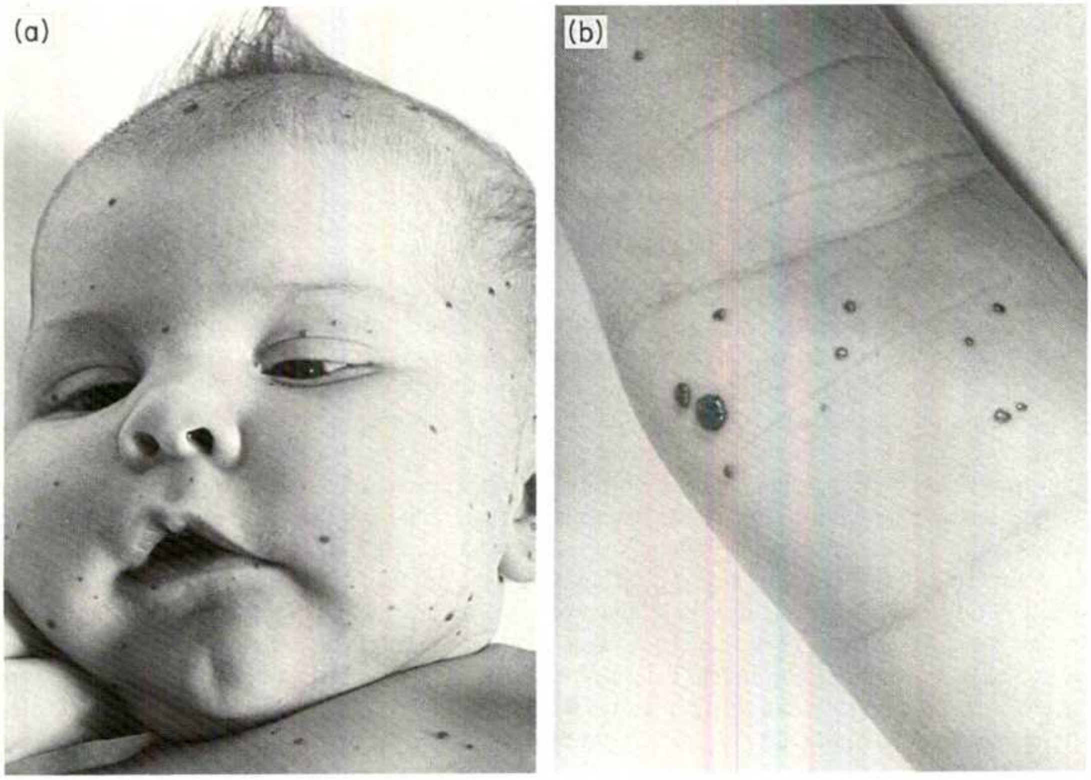

FIGURE 1. Multiple small dome-shaped haemangiomata.

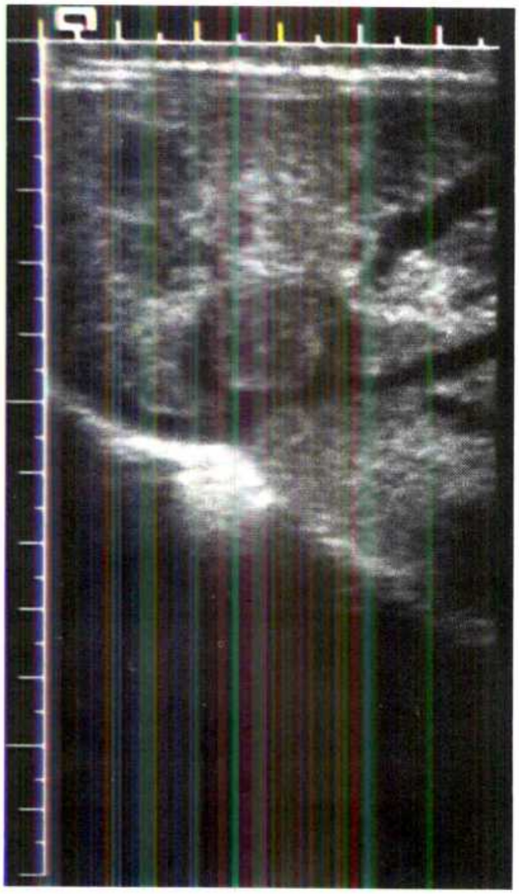

FIGURE 2. Liver ultrasound showing rounded sonolucent areas and enlarged hepatic vein.

less than 5 mm diameter (Fig. 1). All body sites were affected including the oral mucous membrane, and some lesions were noted beneath finger nails. General examination revealed a 3–4 cm smooth-edged hepatomegaly. She was not in heart failure.

Investigations. Liver ultrasound revealed multiple, rounded, sonolucent areas throughout the liver (Fig. 2). Several of these measured 3 cm or more. The hepatic vein was enlarged. No abnormalities were seen in other abdominal organs. Cardiac output was measured at 2 l/min ($=7\cdot8$ l/min/m^2)—slightly elevated. A cranial CT scan showed no abnormalities. Full blood count was normal. From 6 weeks of age, for a month, she was treated with prednisolone 2 mg/kg/day but this did not produce any visible change in the cutaneous haemangiomata and a repeat ultrasound examination of the liver showed no reduction in size of the hepatic haemangiomas.

Comment. In cases of haemangiomas involving the viscera the skin lesions are characteristically eruptive, multiple and small. The most common internal sites to be involved (in order) are the liver, lungs and gastrointestinal tract. The major complications are high output cardiac failure, obstructive jaundice, intestinal obstruction, portal hypertension, vessel rupture giving massive intra-abdominal haemorrhage and platelet-trapping coagulopathy (Kasabach–Merritt syndrome).

Although even large hepatic haemangiomas can regress spontaneously, once complications are evident there is a high mortality, probably about 50%. Approximately 70% of cases treated with prednisolone, and when necessary digoxin, show a measurable response.[2] The skin lesions can give a useful indication of response by becoming soft and grey. This case illustrates the dilemma which occurs when potentially life-threatening visceral haemangiomas are present but have not, at the time of presentation, caused serious complications.

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(7) Swollen toe due to epithelioid haemangioma of bone

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W.C., female, aged 42 years.

History. This patient was referred as a case of 'paronychia'. She had a 6-month history of progressive swelling and some tenderness of the left great toe. She was in excellent general health.

Examination. The toe was diffusely swollen, the pulp was bluish-red in colour and there was increased convexity of the nail (Fig. 1). The circumference was 1 cm greater than that of the right great toe.

Radiographic and magnetic resonance imaging (MRI) features

Plain films. A large lytic lesion of the distal phalanx of the great toe was shown with expansion of the proximal end of the phalanx and an associated large soft tissue mass (Fig. 2). There was no periosteal reaction. No other lesions were apparent.

Radionuclide scans. The whole body static bone scan demonstrated several areas of increased uptake in the left foot and a single lesion in a lower right rib anteriorly. The blood pool phase and static scans of the left foot demonstrated several abnormalities. The blood pool phase (Fig. 3) demonstrated four areas of increased perfusion involving the distal phalanx and metatarsal head of the great toe, the mid tarsus and the region of the medial malleolus. A further possible area of increased perfusion was noted in the region of the talo-navicular joint. The 3-h static scan demonstrated increased uptake in the same four areas, but not in the region of the T-N joint.

Magnetic resonance images. Two scan sequences were performed: a T1-weighted sequence (Fig. 4a), TR = 600 ms, TE = 26 ms; and a STIR sequence (Fig. 4b), TR = 1500 ms, TI = 100 ms, TE = 30 ms. The three anterior lesions demonstrated the same characteristics. On the T1-weighted sequence the findings consisted of a well-defined low signal (grey) area, against a background of high signal (white) normal marrow fat. On the STIR sequence the findings were of a well defined, very high signal (white) central area surrounded by a zone of less well defined and less intense signal against a background of very low signal (black) normal marrow fat. The appearances on the STIR sequence correlate well with the isotope scan appearances. The

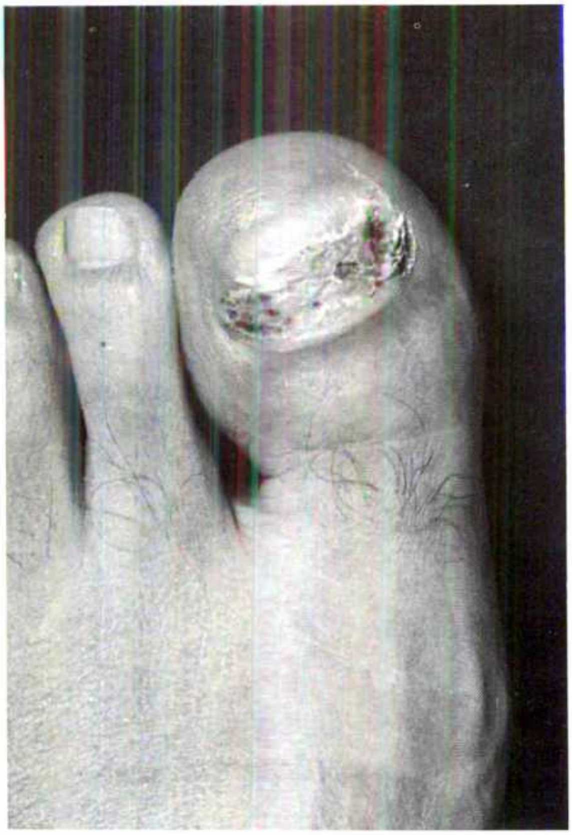

FIGURE 1. Diffuse dusky swelling of left great toe (nail changes are secondary to surgical intervention).

posterior lesion in the talus demonstrated a fairly uniform, but less intense signal than the other lesions on the STIR sequence. No abnormality was seen around the T-N joint.

The MRI scans clearly demonstrated the anatomical site of each of the lesions. The high signal part of the phalangeal lesion clearly involved the distal part of the phalanx, the less intense zone on the STIR sequence involving the proximal part of the bone. The metatarsal lesion was at a subarticular position in the head of the metatarsal. The high signal part of the mid-tarsal lesion involved the lateral part of the lateral cuneiform predominantly, with some involvement of the cuboid. The less intense zone on the STIR sequence involved the lateral cuneiform, the cuboid and the navicular. All these lesions had features to suggest that there was a highly vascular central component with a chronic inflammatory change within the surrounding bone. The posterior lesion, in a subarticular position in the head of the talus, was different from the other lesions. On the T1-weighted sequence there was a well demarcated low signal central area with a thin black line surrounding it and separating it from a slightly higher signal surrounding zone. The STIR sequence demonstrated a central zone that was less intense than the other lesions and a smaller surrounding zone. This could be a small early vascular lesion or an unrelated pathology, such as osteochondritis.

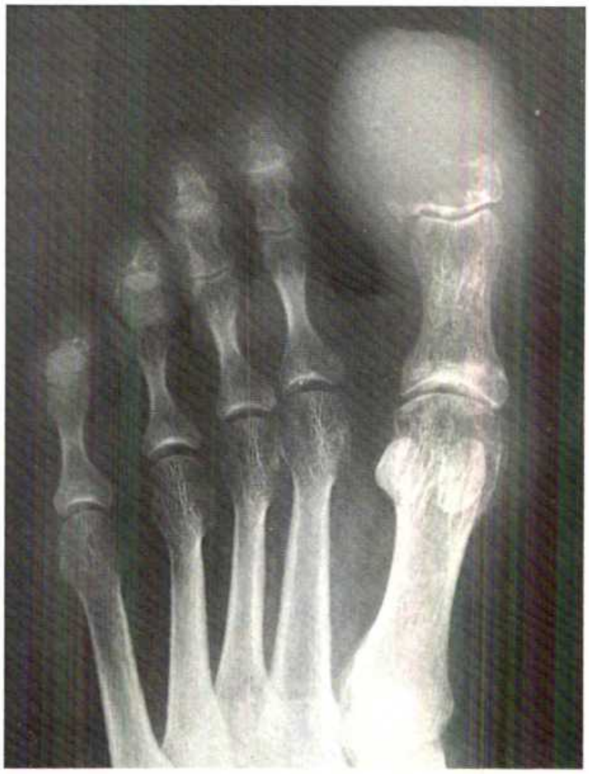

FIGURE 2. Plain film showing lytic lesion.

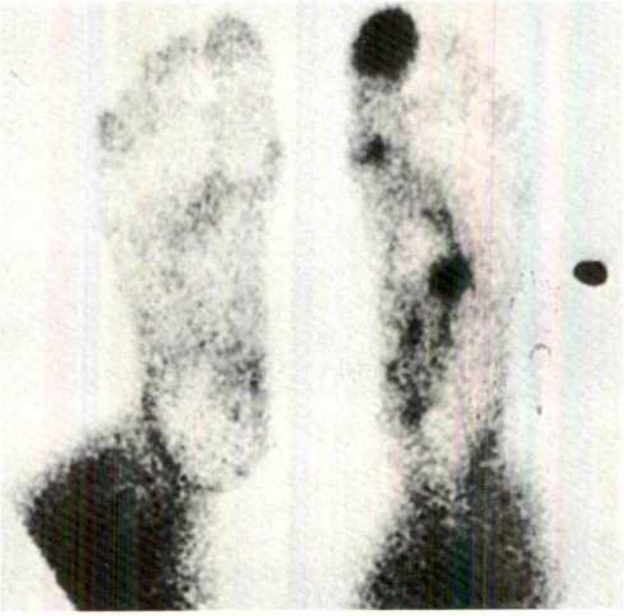

FIGURE 3. Isotope bone scan (blood pool phase) showing multiple lesions.

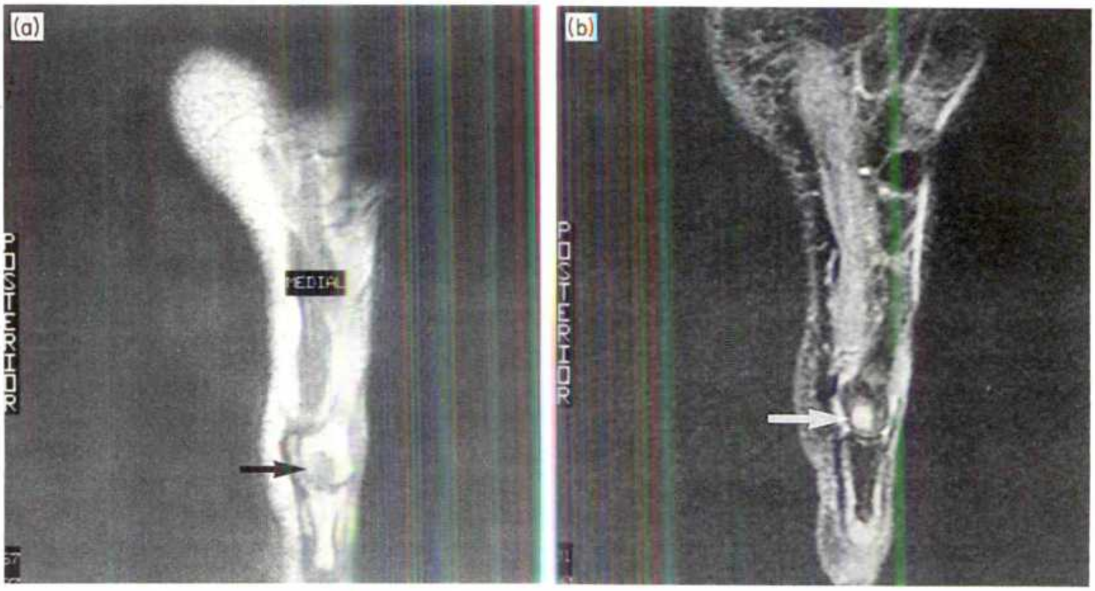

FIGURE 4. The MRI scan: (a) T1-weighted scan, and (b) the STIR sequence.

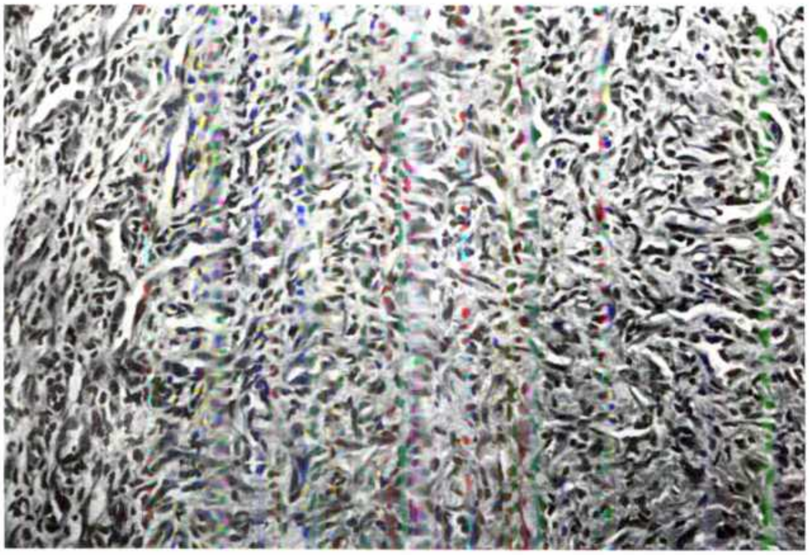

FIGURE 5. High power view of epithelioid haemangioma.

Histopathology. The distal phalanx of the left great toe was explored, and the curetted tissue showed numerous vessels of varying size and development, many with large epithelioid endothelial cells (Fig. 5). Also evident were foci of eosinophils among the associated inflammatory infiltrate.

Comment. Epithelioid haemangioma of bone is a rare vascular tumour. Most cases are multicentric and there is a predilection for the lower extremities. The multicentric cases seem to have a good prognosis and metastasis to extra-osseous sites is rare. Treatment is generally by curettage and/or radiation.

The differential diagnosis on plain X-ray films is wide, including metastatic carcinoma (particularly from a renal primary), aneurysmal bone cyst, giant cell tumour, primary sarcoma of bone and brown tumour, depending on site and multiplicity. The MRI appearances have not been previously described.

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(8) Limb hypertrophy: associated with a solitary plexiform neurofibroma?

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History. An 18-year-old man presented in 1988 with hypertrophy of the right leg, gross varicose veins and a painful enlarging ulcer above the right medial malleolus of 2·5 years' duration.

Increased girth and length of the right leg was first noted at age 4 months and by 9 months several lumps had appeared in front of the right knee and patella. Surgical exploration revealed a

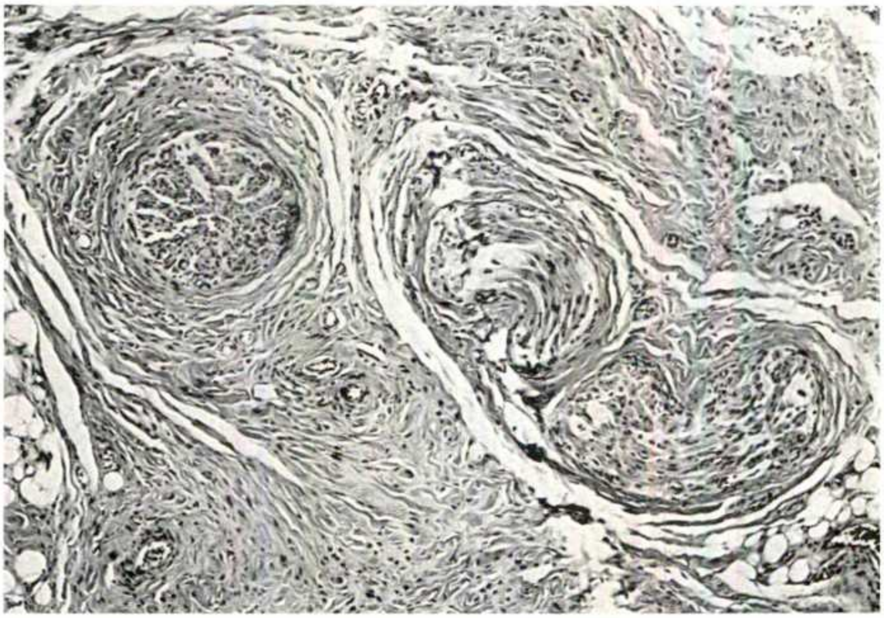

FIGURE 1. Nerves embedded in fibrous tissue (H&E × 125).

subcutaneous mass involving the deep fascia. Macroscopically the irregular nodules appeared non-encapsulated and histologically were reported, at the time, as showing features of a plexiform neurofibroma (Fig. 1).

At age 2 years he developed 'petit mal' which was initially well controlled with primidone, but 2 years later Jacksonian features appeared. EEG showed occasional suspicious paroxysmal features over the right hemisphere but CT scan of the head, air encephalogram and left carotid angiogram were all normal.

By the age of 5 the increased girth of the right leg had become a cosmetic problem, the leg length discrepancy had increased to 4 cm, and there was marked genu valgum. The possibility of surgical intervention was considered but CT scan of the right leg showed all the components of the leg to be massively enlarged, and any reduction in girth would have necessitated the sacrifice of normally functioning muscles. Subsequently, his leg length discrepancy has gradually become less marked.

By the age of 14 prominent varicose veins of the right calf had developed and he underwent stripping of the right long saphenous vein and multiple calf avulsions. One wound on the calf failed to heal and he has suffered intermittent ulceration since then.

Family history. Maternal grandmother and mother have both suffered from epilepsy but there is no family history of neurofibromatosis.

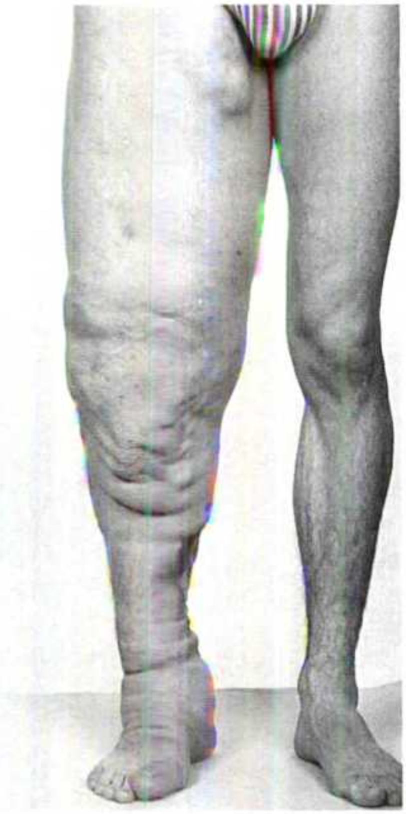

FIGURE 2. Marked hypertrophy of the right leg.

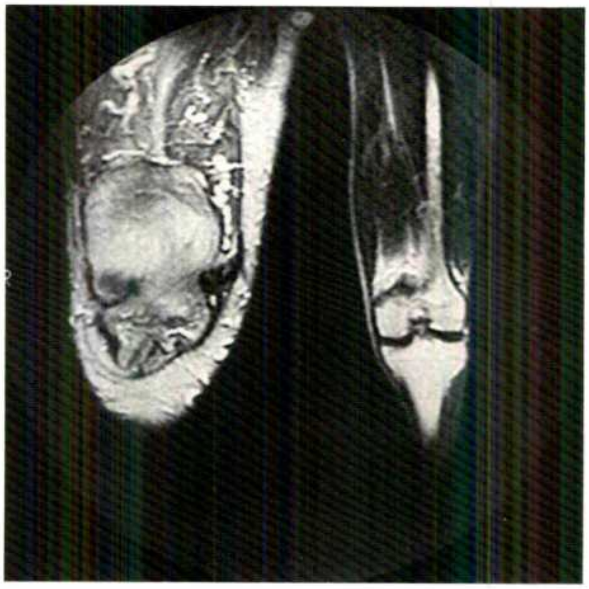

FIGURE 3. Coronal cut of MRI scan showing right suprapatellar mass.

Examination. Marked hypertrophy of the right leg was present (Fig. 2). There was a large varicose ulcer on the medial aspect of the right calf. There were no clinical features of neurofibromatosis.

Investigations. MRI scan of right leg showed hypertrophy of the right leg with enlargement of all components in the thigh and numerous varicose veins. There was no evidence of an arteriovenous malformation. There was a large, well-defined mass in the right suprapatellar pouch (Fig. 3), and abnormal fibrous tissue deposition around tendon insertions at the joint capsule of the right hip and muscle sheaths.

Comment. Elephantiasis-like limb hypertrophy due to a diffuse plexiform neurofibroma is well-recognized in von Recklinghausen's neurofibromatosis.[1] While some authors believe that the presence of a plexiform neurofibroma is itself sufficient to make a diagnosis of neurofibromatosis,[2] Riccardi[1] states that solitary neurofibromas of any type do not automatically warrant a diagnosis of neurofibromatosis, although full evaluation and follow-up is required.

Solitary plexiform neurofibromas, with no other manifestations of neurofibromatosis, appear to be exceedingly rare but have been reported in the following sites: scalp, lumbar region, penis and foot. There is one previous report of limb hypertrophy, manifest by macrodactyly of the foot, associated with a solitary plexiform neurofibroma.[3]

The underlying mechanisms that may produce limb hypertrophy remain unclear. Increased vascularity, direct invasion by neurofibroma cells, mechanical pressure and weight of the tumour together with altered muscle insertion, origins and actions, may be important contributing factors.

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(9) Infantile digital fibromatosis, diagnosed and treated as neurofibrosarcoma 40 years ago

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G.B., female, aged 41 years.

History. At the age of 4 months a nodule developed on the fourth toe of the left foot. It was removed and histologically reported as a simple osteofibroma. A further nodule developed in the scar and the whole toe was amputated. Histology was again reported as simple osteofibroma. A further nodule developed on the fifth toe a few months later and this was also excised. Histopathological opinion was divided but the specimen was considered to be of neural or vascular origin of a low degree of malignancy. Clinically the lesions were judged tumour recurrences and radiotherapy was given, 6000 r over 5 weeks. When she was 4 years old, a new nodule developed on the heel. This was regarded as a low-grade neurofibrosarcoma. Further radiotherapy was given, dosage uncertain.

At age 9, a below-knee amputation was considered because of post-irradiation deformity, recurrent crusting and soreness of the irradiated foot. The surgical notes recorded 'amputation will be necessary sooner or later'.

Comment. The patient has continued to manage with her pain, short limb and deformity and has led an active life without developing further nodules. The whole of the histological material has been reviewed in 1989 (40 years on) and is now considered diagnostic of infantile digital fibromatosis.

(10) Lymphangiectasia of the vulva following hysterectomy and radiotherapy

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J.W., female, aged 41 years.

History. She presented in 1974 with carcinoma *in situ* of the cervix which was treated by cone excision. Subsequent recurrence was treated by Wertheim hysterectomy followed by external 1·5 MeV radiotherapy (5000 cGy over 26 treatments). Histopathology showed a poorly differentiated squamous cell carcinoma of the cervix but there was no lymphatic invasion or lymph node involvement. After treatment she had slight, non-progressive leg oedema. She was seen in 1989 with a 5-year history of blistering of the vulva with leakage of fluid. Coincidental medical history included pernicious anaemia, hyperthyroidism and fibrosing alveolitis.

Examination. There was lymphoedema of the vulva with small, tense, clear, fluid-filled vesicles.

Histology. A skin biopsy showed dilated thin-walled vessels in the superficial dermis.

Comment. Dilatation of cutaneous lymphatics producing an appearance that resembles lymphangioma circumscriptum has been described after resection of regional lymph nodes,[1] after radiography,[2,3] and after both forms of treatment for carcinoma of the cervix.[4]

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(11) Lymphangiomata following surgery and radiotherapy for breast carcinoma

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Case 1

History. A 65-year-old female presented in October 1988 with a 12-month history of multiple, reddish-purple lesions over the lower aspect of the left breast and adjacent chest wall. Stage I carcinoma of the left breast had been diagnosed in July 1985 and treated by 'lumpectomy' and post-operative radiotherapy (4700 cGy tumour dose left breast, 4692 cGy tumour dose left axilla, 20 fractions in 35 days, 900 cGy applied dose to scar, 5 fractions in 4 days).

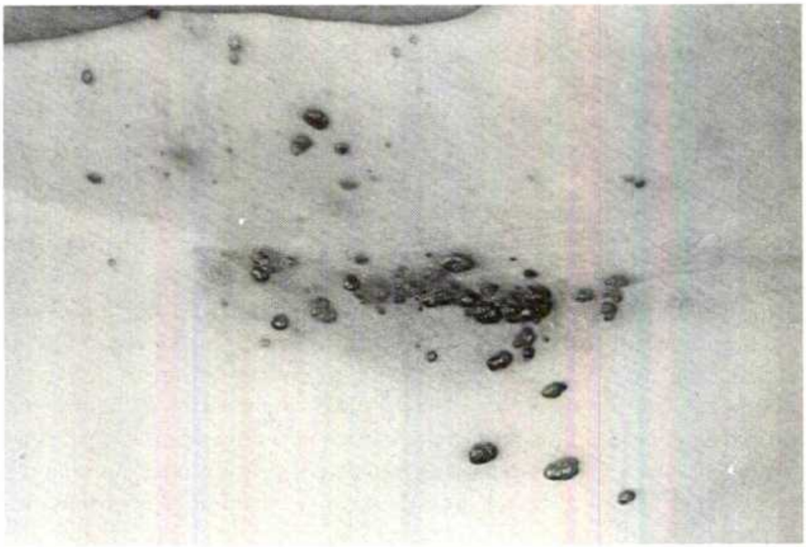

FIGURE 1. Lymphangiomata: multiple vesicles on and beneath the left breast.

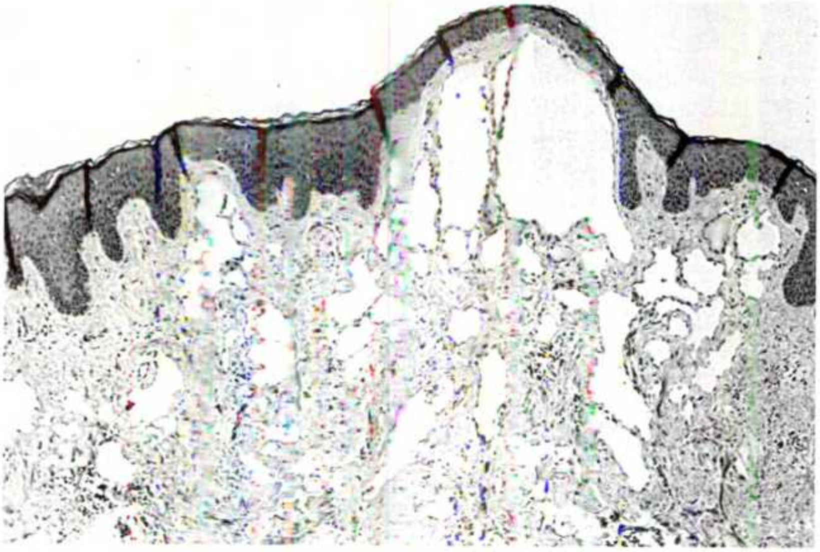

FIGURE 2. Ectatic lymphatic spaces in the papillary dermis (H&E × 163).

Examination. There were 40–50 reddish purple vesicles over the lower aspect of the left breast and adjacent chest wall (Fig. 1). Some of the vesicles were surrounded by a purple halo and a number of the lesions showed a linear distribution in relation to a previous biopsy scar. There was no clinical evidence of radiodermatitis, tumour recurrence, lymphadenopathy or lymphoedema.

Histology. Lymphangioma composed of large sinusoids lined by endothelium (Fig. 2).

Case 2

History. A 76-year-old lady presented in June 1989 with a reddish-purple confluent lesion on the left side of the chest wall. Carcinoma of the left breast, diagnosed in December 1976, had been treated with extended simple mastectomy and post-operative radiotherapy (4496 cGy tissue maximum dose to the chest wall and 3200 cGy applied dose to the intermammary chain, axilla and supraclavicular fossa, 16 fractions in 21 days).

Examination. Reddish-purple fluid-filled lesion 10 × 5 cm in diameter on the left chest wall, medial to the mastectomy scar. There was no evidence of tumour recurrence or lymphadenopathy.

Histology. Lymphangioma with an endothelial lining, surrounded by a foreign body giant cell response.

Comment. Acquired lymphangioma can be indistinguishable clinically and histologically from lymphangioma circumscriptum. It is a rare but well-recognized complication of chronic lymphoedema and has been previously reported following mastectomy, mastectomy with radiotherapy, and radiotherapy of the vulva. Lymphatic obstruction is the presumed cause and it appears to be extremely rare in the absence of clinical lymphoedema. So far as we know, there are no reported cases of lymphangiomata occurring following 'lumpectomy' and radiotherapy. Lymphangiosarcoma of Stewart and Treves is a more commonly reported complication of mastectomy than benign lymphangioma. There are no reports of benign acquired lymphangiectasia progressing to a malignant form.

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(12) Differentiation between pseudolymphoma and malignant B-cell lymphoma of the skin

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Case 1

History and examination. A 66-year-old man presented in June 1984 with a 6-month history of itching and burning papules which had started on the right temple and spread to involve the forehead. He had suffered from ankylosing spondylitis since the age of 30. There was no past history of radiation therapy. Examination revealed translucent erythematous papules and nodules on the right temple and forehead adjacent to the scalp margin. His spine was ankylosed.

Investigations. Full blood count, plasma viscosity and a chest radiograph were normal. A Kveim test was negative. Histology of a skin biopsy showed foci of lymphoid cells in the mid dermis with reactive germinal follicles and a surrounding granulomatous reaction.

Treatment. A diagnosis of lymphocytoma cutis was made and topical treatment with fluocinolone acetonide 0·025% resulted in regression of the lesions.

Course. He was lost to follow-up but was referred back in October 1987 with a further crop of lesions on his right temple of 9 months' duration. A mass on the left side of his neck had been noted 5 months earlier and had gradually enlarged. On examination there were erythematous papules and nodules over the right temple (Fig. 1) and hard left cervical lymph nodes were

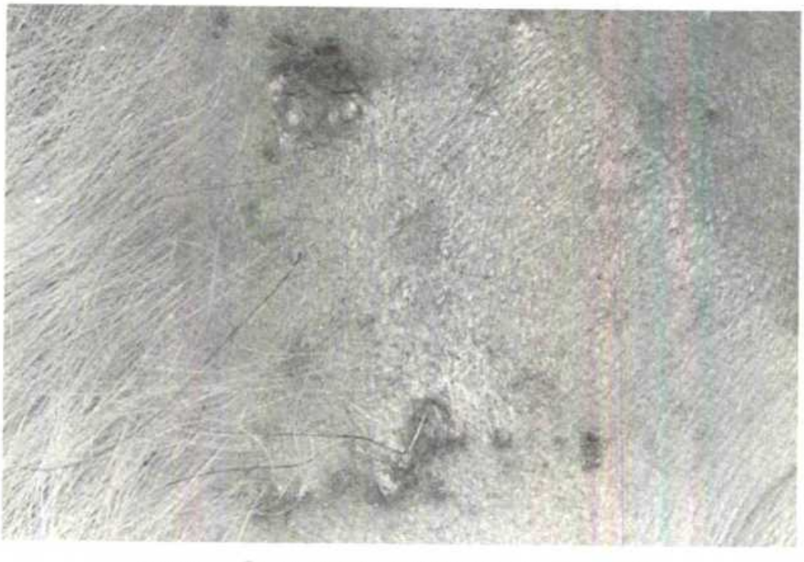

FIGURE 1. Case 1: papules and nodules on right temple.

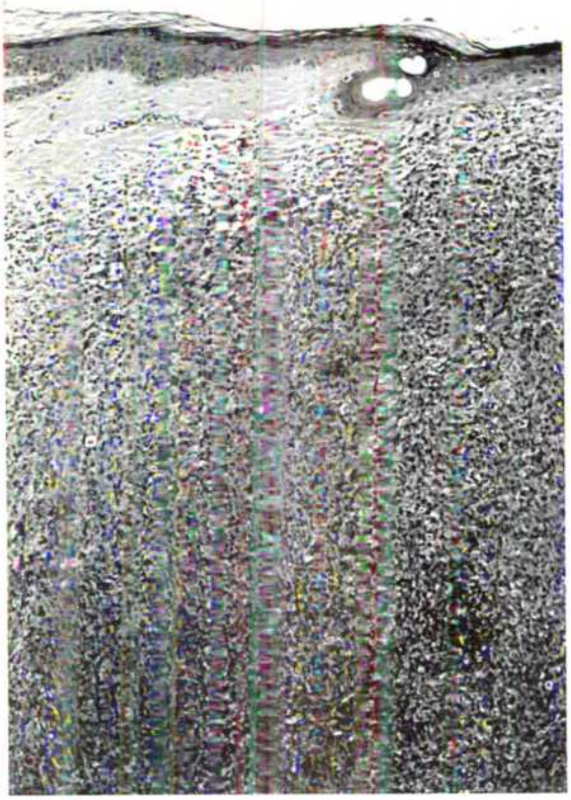

FIGURE 2. Case 1: skin biopsy showing nodular but monomorphic infiltrate in the dermis (H&E × 125).

palpable. There was no hepatosplenomegaly. CT scans of chest and abdomen were normal. Lymph-node biopsy showed centroblastic/centrocytic non-Hodgkin's lymphoma and a further skin biopsy also showed nodular aggregates of non-Hodgkin's lymphoma within the dermis (Fig. 2).

He initially received 1200 cGy applied dosage of radiotherapy (250 KV), four treatments in 3 days, to the left-hand side of his neck but, in view of a persistent mass in the left-hand side of his neck and a small node on the right, a further 900 cGy were applied to the left (three fractions in 2 days) and 1500 cGy (five treatments in 4 days) to the right in May 1988 with a good response. The skin lesions responded to intralesional triamcinolone and topical clobetasol propionate 0·05%. In December 1988 a further enlarging painful lump (10 × 5 cm in diameter) appeared on the left-hand side of his neck and he was commenced on palliative chemotherapy in the form of oral chlorambucil and prednisolone. He responded initially but his condition subsequently deteriorated and he died at home in June 1989.

Case 2

History. A 66-year-old lady presented in February 1987 with a lesion on the left temple of 6 months' duration. Examination revealed a 1-cm diameter erythematous lesion in the left temple region. The lesion was excised and histology showed large aggregates of lymphoid cells

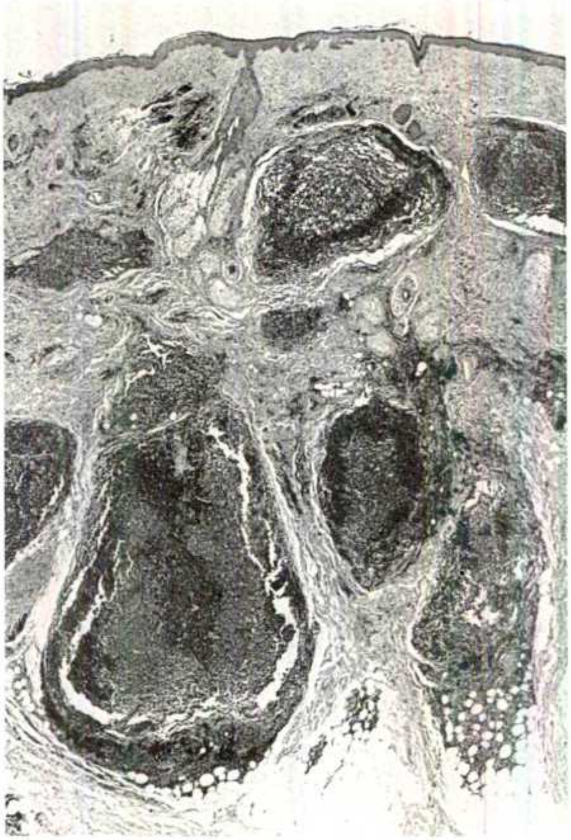

FIGURE 3. Case 2: deep, 'bottom heavy', follicular infiltrate in the dermis (H&E × 35).

throughout the dermis with extension into the subcutaneous tissue (Fig. 3). There was some follicle formation but the infiltrate was 'bottom heavy'.

In September 1987 a small erythematous nodule adjacent to the previous scar appeared and gradually enlarged (Fig. 4). Over the next 18 months four itchy erythematous nodules developed on the lower back. There was no lymphadenopathy or hepatosplenomegaly. CT scans of chest and abdomen were normal. Skin biopsies were taken of the recurrent nodule on the left temple and a nodule on the back. These revealed aggregates of small lymphoid cells in the dermis, with infiltration of the cells between the collagen bundles. Bone marrow examination was normal.

Comment. Differentiation between pseudolymphoma and malignant B-cell lymphoma of the skin may be difficult. A number of distinguishing features have been previously described and these are listed in Table 1.

The histology of the original lesion in Case 1 showed the typical appearances of lymphocytoma cutis and yet this patient developed a high grade and fatal lymphoma. The presence of a 'bottom heavy' infiltrate extending into the subcutaneous tissues in the second case is suggestive of a malignant B-cell lymphoma. Staging and follow up over 3 years has, however, revealed no evidence of systemic involvement, so far.

TABLE 1. Adapted from Burg G *et al.*[3]

	Pseudolymphoma	Malignant B-cell lymphoma
Skin lesions		
Number	Usually solitary	Usually multiple
Preferential anatomical site	Head and neck	No predilection
Histology		
Architecture of infiltrate	Usually well circumscribed, wedge-shaped with sharply demarcated germinal centres	Usually poorly demarcated and 'bottom heavy'
Composition of infiltrate	Predominantly polymorphous with many macrophages	Predominantly monomorphous with few macrophages

Recently the presence of light chain restriction using immunohistochemical techniques has been used to demonstrate clonality of B cells. Studies in these two patients have, however, been unhelpful. Genomic analysis to show either rearrangement of the immunoglobulin genes or 14:18 translocation may be helpful, but is probably impractical in routine clinical practice.

Bafverstedt[1] states that lymphocytoma cutis may rarely undergo malignant change and Halevy and Sandbank[2] report malignant transformation associated with the sign of Leser–Trélat. Others, however, argue that in such cases the original 'pseudolymphoma' was probably malignant from the start.

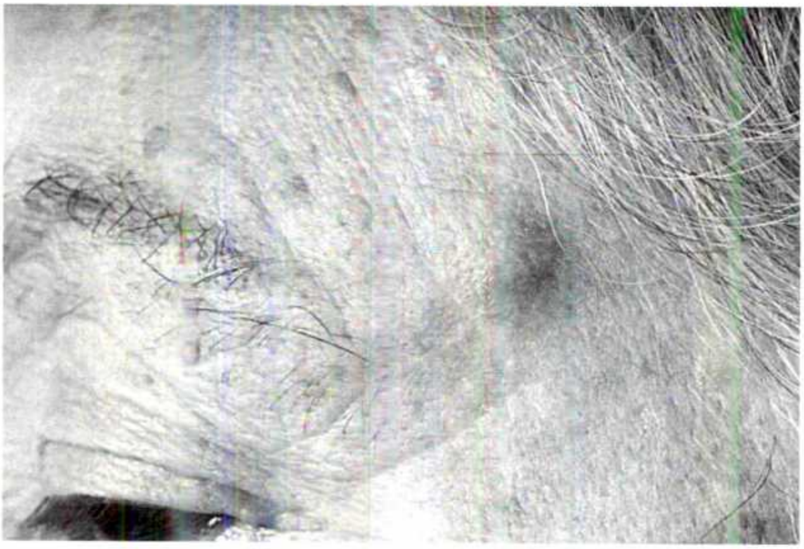

FIGURE 4. Case 2: solitary nodule on left temple.

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(13) Lymphomatoid papulosis progressing to high grade lymphoma

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J.M., male, aged 58 years.

History. This 58-year-old radiographer developed lymphomatoid papulosis in 1961. Since then the condition has run a fairly benign course and during 1983 cleared with PUVA for a year. Unfortunately, treatment had to be discontinued because he developed severe PUVA pain. The lymphomatoid papulosis then recurred.

In the autumn of 1987, he presented with typical lesions of lymphomatoid papulosis on his trunk and limbs but, in addition, he had a very atypical ulcerated lesion over the left lower thigh measuring some 4 cm in diameter. Four lymphomatoid papulosis lesions had coalesced to form a larger lesion, biopsy of which was reported as lymphomatoid papulosis. The lesion failed to heal spontaneously and was treated with low dose superficial X-rays. This initially produced modest improvement but soon after the lesion continued to enlarge.

It was apparent that the lesion was behaving in a malignant fashion and he was referred to the Skin Tumour Unit at St. John's Hospital. Further biopsy showed a transformation to high

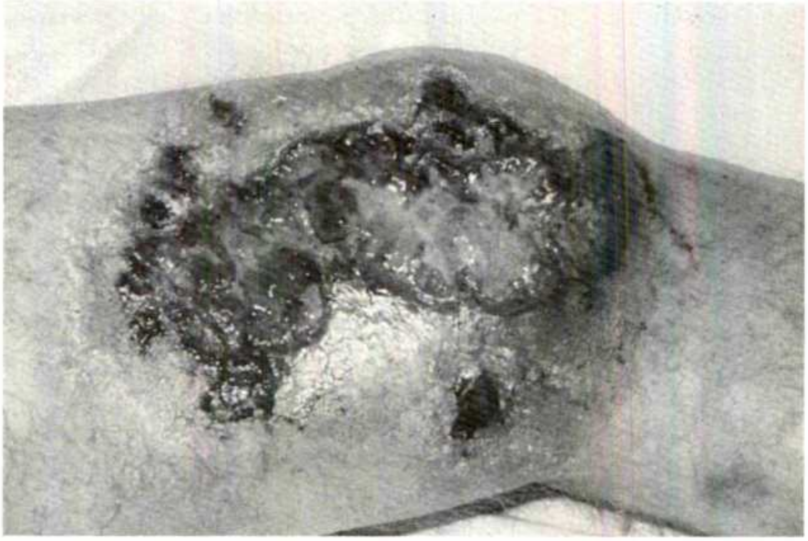

FIGURE 1. High grade B-cell lymphoma arising from medial aspect of left lower thigh and knee.

grade B-cell lymphoma. He was treated with radiotherapy (2100 cGy) left thigh and (1600 cGy) to the inguinal lymph nodes and the lesion healed completely. A further crop of smaller lesions recurred on the thigh proximal to the main lesion some months later. These resolved satisfactorily with further radiotherapy.

Examination. He had an 8 × 12 cm ulcer on the left medial aspect of the left lower thigh extending towards the knee (Fig. 1). He had 'shotty' nodes in the left inguinal region. There was no hepatosplenomegaly and examination was otherwise normal.

Investigation. Full blood count, routine biochemistry and left inguinal lymph node biopsy were normal. CT scan of abdomen and groin showed no evidence of metastatic lymphoma.

Comment. Lymphomatoid papulosis is generally considered to run a benign course but progression to lymphoma is recognized.[1]

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(14) Adult T-cell leukaemia/lymphoma

Dr Susan C.Parker, Dr S.Whittaker, Dr N.Smith, Dr M.Spittle and
Dr R.A.J.Eady

St. John's Hospital, London

A 30-year-old woman presented in July 1989 with a 10-week history of a widespread itchy papular eruption which subsequently became more infiltrated, particularly on the face. Her parents were West Indian but she had lived all her life in the U.K. It was subsequently discovered that her sister had died of adult T-cell leukaemia/lymphoma (ATLL) aged 31, and that both her parents were HTLV1 positive.

Investigations showed that HTLV1 antibodies were positive in our patient and that there were abnormal clefted T lymphocytes present on the blood film and in the bone marrow. Serum calcium was normal and there was no evidence of lymphadenopathy. Skin biopsy revealed a lymphomatous infiltrate in the dermis. These abnormal T cells were clonal and contained HTLV1 proviral DNA.

Our patient has been commenced on combination chemotherapy (CHOP) and has so far done very well with clearance of skin and bone marrow. Unfortunately, as median survival in this condition is less than 1 year, her prognosis is likely to be poor.

Comment. Cutaneous involvement such as in our patient occurs in about 50% of patients with ATLL. Lymphadenopathy and hypercalcaemia occur even more commonly. Unusually, our patient has developed neither of these as yet. Most patients are seen in the West Indies and Japan. At the current time, ATLL is rare in the U.K. with fewer than 100 individuals being confirmed as sero-positive for HTLV1, and fewer than 20 having developed ATLL.

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(15) Erythema nodosum and cutaneous vasculitis associated with recurrence of Hodgkin's disease

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History. A 51-year-old man presented in 1981 with a 6-week history of a submandibular swelling. Examination revealed a 4 × 3 cm mass superficial to the submandibular salivary gland with cervical and axillary lymphodenopathy. Biopsy of the submandibular mass showed mixed cellularity Hodgkin's disease which was staged as IIIA. He responded well to seven courses of cyclical chemotherapy (chlorambucil, vincristine, procarbazine and prednisolone—LOPP) and went into complete remission.

He was well until June 1988 when he developed raised, red, annular lesions on his upper arms, conjunctival redness, red painful lumps on both shins and peripheral joint pains. Two weeks later confluent erythematous plaques over the dorsum of the hands and fingers appeared.

Examination. There were raised erythematous annular lesions most prominent on the upper arms (Fig. 1), but extending onto the forearms with confluent plaques over the dorsum of the hands and fingers. There was bilateral erythema nodosum of the lower legs, a symmetrical

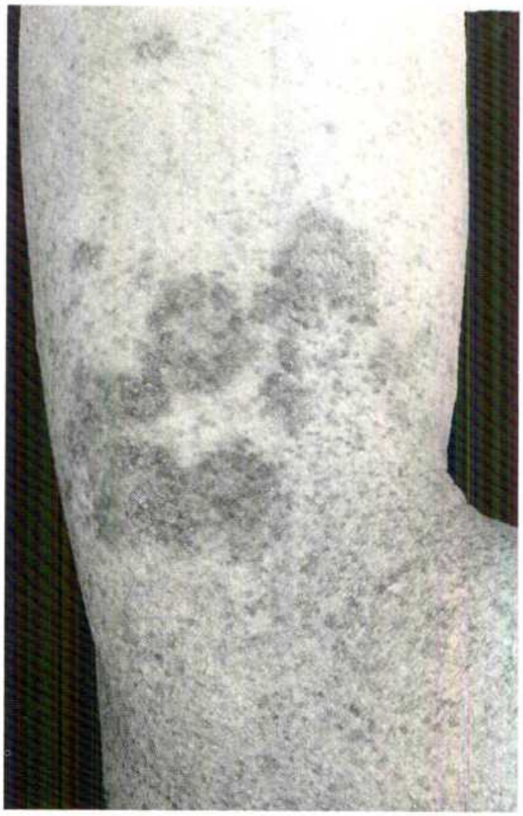

FIGURE 1. Raised annular lesions on right upper arm.

arthritis affecting the wrist and ankle joints and scleritis. There was no lymphodenopathy or hepatosplenomegaly.

Investigations. Haemoglobin 10·5 g/dl, white blood cell count 17·7 × 10^9/l (granulocytes 14·1 × 10^9, lymphocytes 1·9 × 10^9, monocytes 1·8 × 10^9), platelet count 724 × 10^9/l, plasma viscosity 1·82 cp (normal range 1·5–1·72). The following investigations were normal or negative: liver function tests, autoimmune profile, complement studies including studies for circulating immune complexes, serum angiotensin converting enzyme, anti-streptolysin 'O' titre, anti-streptococcal DNAse B, Kveim test, chest X-ray and CT scan of abdomen.

Histology. Several skin biopsies taken from the upper limbs showed similar histological features with superficial dermal oedema, plump reactive endothelial cells, and vascular ectasia in the superficial vascular plexus. In the deeper vascular plexus there was evidence of a vasculitis with some leucocytoclasis. Direct immunofluorescence was negative.

Progress. The erythema nodosum responded to ibuprofen 400 mg t.d.s. but his systemic symptoms and the rash persisted for several weeks. Prednisolone 30 mg daily was consequently introduced with marked improvement in all symptoms, but a relapse occurred when the dosage was reduced to 5 mg daily. Dapsone also produced symptomatic relief but was stopped when an anaemia of 9·4 g/dl occurred. In November 1988, 6 months after the onset of the rash, bilateral cervical and left axillary lymphadenopathy was noted and histology of a node biopsy showed recurrent Hodgkin's disease. He received six further courses of LOPP chemotherapy. Initially his rash would disappear during the courses of chemotherapy but would recur between such courses. During later courses the periods of remission became longer and his rash subsequently cleared completely. In August 1989 he received a course of radiotherapy (3000 cGy tumour dose, 6 MeV, 10 treatments in 13 days) because of persistent nodes in the left supraclavicular fossa and left axilla.

Comment. The diagnosis of this man's upper limb rash has been the subject of considerable discussion. Sarcoidosis and granuloma annulare were initially considered, but investigations failed to provide supporting evidence and histology showed leucocytoclastic vasculitis. Apart from brief remissions induced by systemic steroids and dapsone, the vasculitis persisted for several months and preceded clinical evidence of recurrence of his Hodgkin's disease. Although the brief remissions of the vasculitis during the initial courses of chemotherapy may be attributable to oral steroids, the subsequent induction of a prolonged remission suggests a true association between cutaneous vasculitis and Hodgkin's disease in this patient. Erythema nodosum[1] and cutaneous leucocytoclastic vasculitis[2,3] have independently been reported in association with Hodgkin's disease.

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(16) Nodular non-Langerhans cell histiocytosis

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I.G., female, aged 50 years.

History. In 1982 this woman developed non-tender lesions on her trunk and limbs (Fig. 1). These began as papules and over a 2-year period became more extensive and nodular. The lesions have progressed over the last 8 years and, in places, have become confluent although somewhat flatter.

Examination. She has a nodular purple-red eruption over her trunk and limbs extending to her neck and face. Her hands and feet are relatively spared. There is no hepatosplenomegaly or lymphadenopathy.

Investigations. Full blood count, autoimmune profile, cholesterol and triglycerides, immunoglobulins and protein electrophoresis were all normal. X-ray of chest and pituitary fossa was normal.

Histology. A number of biopsies have been performed and all show essentially similar features: an upper and mid-dermal infiltrate of unusual appearing histiocytes, Touton giant cells and xanthoma cells. The histiocytes showed a granular eosinophilic cytoplasm. S100 stain was negative and Birbeck granules were not seen on electron microscopy.

Comment. The eruption began 3 months after a blood transfusion for postpartum haemorrhage. The patient is convinced that this was the cause of her skin eruption and has been very reluctant to consider any treatment capable of producing side-effects. She had a course of steroids and, more recently, she agreed to short courses of azathioprine and cyclosporin, all without benefit. Her latest treatment was with etoposide but she has now refused to continue treatment.

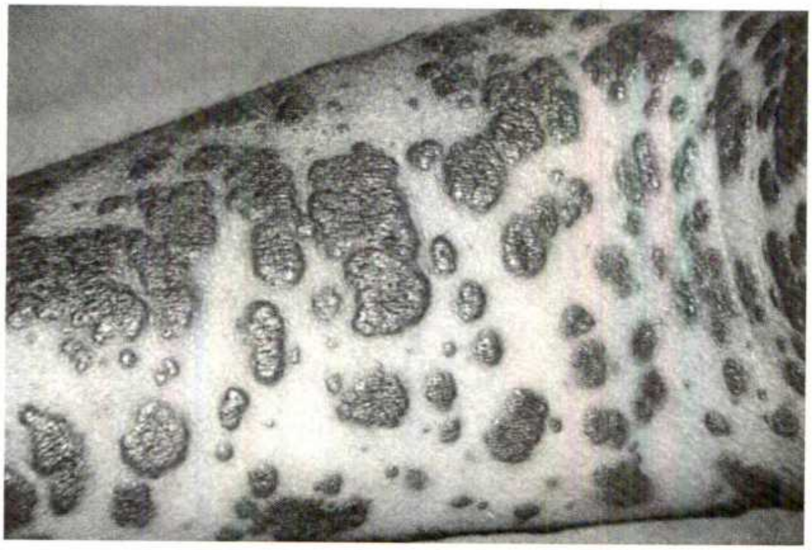

FIGURE 1. Nodular non-Langerhans cell histiocytosis: purple-red nodules of forearms.

Her condition may be best classified as normolipaemic nodular non-Langerhans cell histiocytosis and is similar to the case described by Prendiville *et al.*[1]

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(17) Wegener's granulomatosis presenting with blepharo-conjunctivitis

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E.P., female, aged 67 years.

History. This patient presented in 1988 with acute bilateral blepharo-conjunctivitis and a localized ulcerated nodule on the left lower eyelid measuring 1·5 cm in diameter. Apart from a longstanding rheumatoid nodule on her right elbow, her skin and mucous membranes were normal. Her chronic rheumatoid arthritis was quiescent on treatment with Diclofenac.

The clinical differential diagnosis was cicatricial pemphigoid, pyoderma, vasculitis or lymphomatoid papulomatosis.

Investigations. Hb 13·7 g/dl, leucocytes 3100/mm^3, ESR 31 mm/h, rheumatoid factor positive at 1:20, immunoglobulins normal, bacteriology negative. Biopsy of the eyelid nodule showed collections of atypical pale cells in the dermis, loosely arranged around blood vessels extending between collagen bundles. There was considerable cell atypia with numerous mitotic figures, some of which were atypical and a neoplastic process was suspected (Dr Neil Smith).

Course. The eye lesions resolved after treatment with topical steroids and systemic pivampicillin. In November 1988, she developed a painful swelling of her left cheek following a knock. She was referred to the ENT Department where examination revealed an extensive firm mass arising from the front wall of the maxillary antrum with weakness of the left upper lip. The skin overlying this showed purple discolouration but no ulceration. There was no lymphadenopathy, and the nose was clear. In December 1988, she was pyrexial with an indurated mass of the left maxilla ulcerating through the hard palate (Fig. 1). There was splenomegaly but no hepatomegaly.

Investigations

Hb 11·4 g/dl, WBC 2400 mm^3, platelets 171,000 mm^3, ESR 25 mm/h, blood urea 4·3 mmol/l, bilirubin 7 μmol/l, AST 28 IU/l, SGGT 122 IU/l, alkaline phosphatase 150 IU/l, albumin 31 g/l, bone marrow normal, neutrophil cytoplasmic antibodies negative.

CT scan of the face in December 1988 showed bone destruction of the medial wall of the antrum as well as thickening, consistent with Wegener's granulomatosis.

Chest X-ray showed ill-defined soft shadowing of right middle lobe.

Histology. A biopsy of the antral mass showed a necrotizing granulomatous process with few lymphocytes and plasma cells, many neutrophils and histiocytes. There were two tiny granulomas with Langhans-type giant cells. Vasculitis was seen in areas away from the necrosis. Conclusion: 'Wegener's granulomatosis' (Dr Ng).

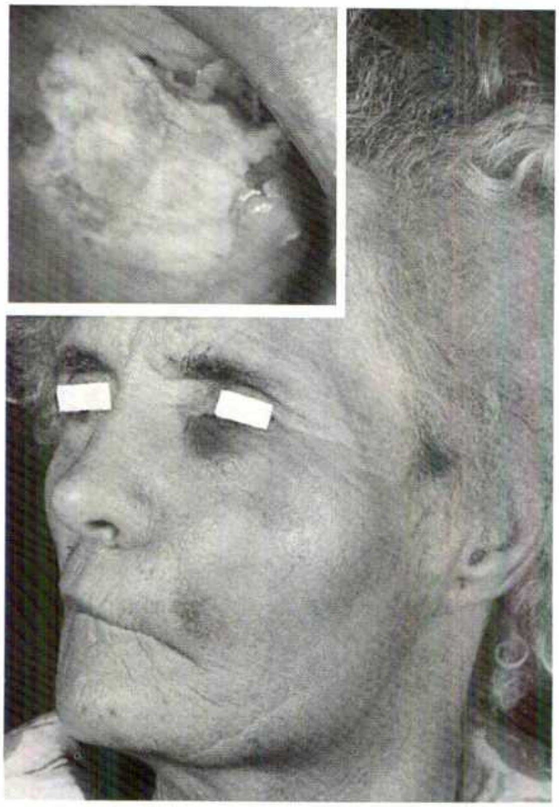

FIGURE 1. Facial swelling in Wegener's granulomatosis. Insert shows ulceration through hard palate.

Progress. Treatment was started with prednisolone 40 mg daily combined with cyclophosphamide 100 mg on alternate days. There was initial improvement but in early March 1989, her condition deteriorated, and she again developed fever, lethargy, signs of bronchopneumonia and leukopenia. Despite stopping cyclophosphamide, and starting antibiotics, she continued to deteriorate, her renal and hepatic function worsened, and she died in April 1989.

Comment. The histology of this patient's antrum together with lesions involving both the upper and lower respiratory tract fulfil the criteria for the diagnosis of Wegener's granulomatosis;[1] although there was no evidence of direct renal involvement and tests for neutrophil cytoplasmic antibodies were negative.

Our patient was unusual in presenting with non-specific skin and eye lesions. She demonstrated the problems encountered in the diagnosis of this multisystem disease. Combined treatment with cyclophosphamide and prednisolone has been reported to produce a long-term remission in 93% of 85 patients with Wegener's granulomatosis,[1] but this treatment was unsuccessful in our patient.

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(18) Sarcoid-like reaction: case for diagnosis

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S.V-C., male, aged 30 years.

History. This publican in 1986 sustained a chemical burn to his right hand while cleaning beer pipes with sodium hydroxide. A hole in his gloves allowed the sodium hydroxide solution to come in contact with the skin over the base of the right thumb and first finger and the resultant injury was slow to heal. He developed some numbness and discomfort and swelling at the site which did not respond to treatment with several courses of antibiotics and analgesics. His symptoms became so severe that prednisolone 60 mg on alternate days was introduced with considerable improvement in his symptoms and reduction in the swelling. However, it was impossible to reduce the dose of prednisolone because of rapid recurrence of his symptoms.

In the spring of 1989, he developed a scaly eruption over his arms, trunk and face with the development of more discrete lesions suggestive of pityriasis lichenoides on his upper arms, shoulders and face. His daily dose of prednisolone at the time was 40 mg. Histology of the scaling lesions showed granulomata and the more discrete lesions were suggestive of lymphomatoid papulosis. This eruption cleared spontaneously and has not recurred.

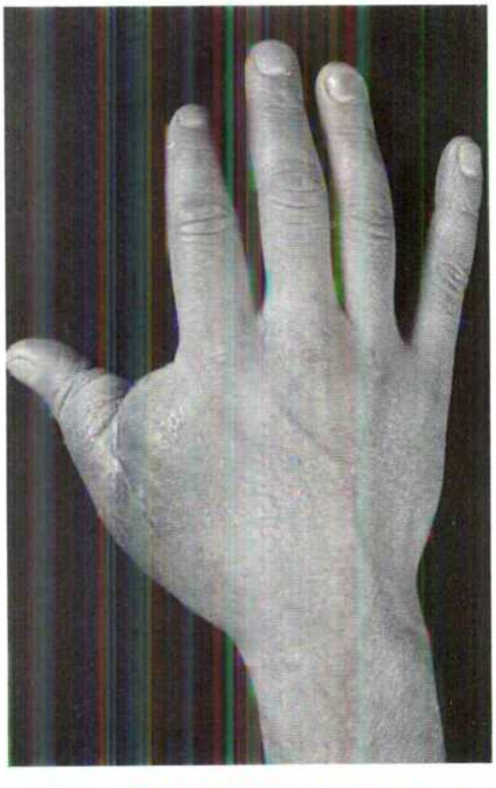

FIGURE 1. Right hand showing considerable swelling of the base of the right thumb and thenar eminence.

Examination. He now has considerable swelling over the right thumb, base of the thumb and thenar eminence (Fig. 1). There is no lymphadenopathy or hepatosplenomegaly.

Histology. Histological examination showed very extensive disruption of soft tissue and muscle by a profound inflammatory process. Epithelioid cells and giant cells of both Langhan's and Touton type were present with a heavy lymphoid infiltrate. Eosinophils and acute inflammatory cells were virtually absent. Moderate numbers of plasma cells were present.

Investigation. Routine haematology and biochemistry, culture for fungus, tests for sexually transmitted disease, SACE, chest X-ray and CT scan of abdomen were all negative or normal. Kveim test was attempted but invalidated because the patient could not remain off systemic steroids for 6 weeks. MRI and X-ray of the hand have shown soft tissue swelling with no bony involvement.

Comment. What was the significance of the initial injury with sodium hydroxide? Histology showed sarcoid-like features but there was no other evidence of sarcoidosis. Currently, the dose of prednisolone is being reduced and methotrexate 20 mg weekly has been introduced with some initial improvement.

(19) Necrolytic migratory erythema: apparent response to oral omega-3 (marine) essential fatty acids

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D.P., female, aged 46 years.

History. Diabetes was diagnosed in 1984 and was controlled initially by diet then oral hypoglycaemics; it became increasingly difficult to control with insulin in 1987. She also

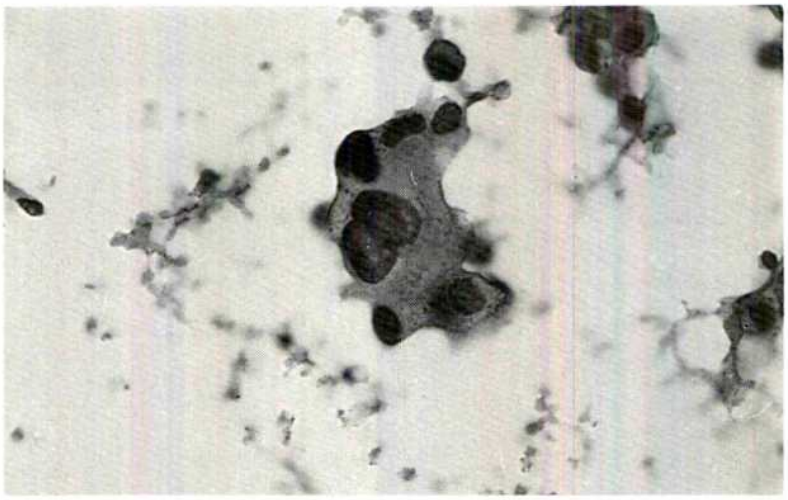

FIGURE 1. These malignant cells from the metastatic tumours in the liver show nuclear pleomorphism and positive staining of the neurosecretory granules in the cytoplasm with the Bodian stain.

developed inflammatory reactions at the sites of insulin injections. In 1986 she began to suffer from dyspepsia diagnosed on endoscopy as gastritis and oesophagitis. In early 1988 she presented with a widespread uncomfortable rash affecting particularly the legs, feet, buttocks, abdomen, and hands. In addition she had a marked glossitis and cheilitis.

Examination. Widespread tender erythematous macules were present in the affected areas, confluent in places. Some patches were psoriasiform, others showed central erosion and peripheral spread, the pattern slowly changing from week to week. Marked emotional lability and depression were apparent.

Investigations. Ultrasound and CT scans demonstrated a soft tissue mass in the tail of the pancreas extending to the hilum of the spleen. In addition, there were areas in the liver consistent with multiple metastases. She had an iron deficiency anaemia, slightly raised ALT, and normal zinc levels.

Glucagon estimation (Professor S.R.Bloom, Hammersmith Hospital) showed raised levels at 141 pmol/l plasma (normal less than 50). Gastrin was also raised at greater than 400 pmol/l (normal less than 40).

Aspiration needle biopsy of the liver confirmed the presence of malignant cells which were compatible with an islet cell tumour origin (Fig. 1).

Histology. Early skin lesions showed the typical appearances of necrolytic migratory erythema. The most striking feature was abrupt necrolysis affecting the upper third of the epidermis (Fig. 2), the necrolytic cells having amorphous eosinophilic cytoplasm and pyknotic nuclei. Numerous small vesicles were present throughout the necrotic zone with early blister formation. The granular layer was absent and a patchy infiltrate of polymorphs was present. Older lesions showed less specific changes consisting of epidermal thickening with a parakeratotic crust.

Progress. She was treated with topical steroids and emollients, a high protein diet and zinc supplements, plus courses of streptozotocin and 5 fluoro-uracil. Unfortunately, she was unable to tolerate chemotherapy and became upset and uncooperative, eventually refusing further

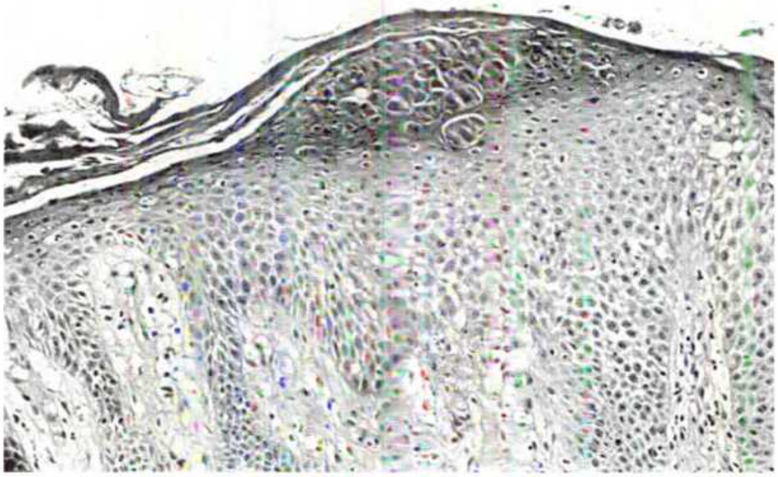

FIGURE 2. In the upper epidermis there is abrupt necrolysis with early blister formation.

treatment. Symptomatic treatment with ranitidine relieved her dyspepsia, but her skin and general health deteriorated. By late 1988 her liver had become palpably enlarged and tender, and a repeat CT scan confirmed increase in size of the metastases. Plasma glucagon and gastrin levels remained elevated.

Omega-3 marine triglycerides orally (Maxepa: Duncan Flockhart: 10 capsules daily) were commenced empirically in December 1988. Rapid and virtually complete clearing of the skin lesions followed, with marked improvement in her general well-being and psychological state. Somatostatin, tried in October 1989, was not tolerated because of nausea. She remains (January 1990) reasonably well, but with increasing liver enlargement and uncontrolled diabetes. Her skin remains clear and she continues to take Maxepa 10 capsules daily.

Comment. This patient was much distressed by widespread necrolytic migratory erythema due to a pancreatic glucagonoma with hepatic metastases. Her rash was severe and failed to respond to standard management.[1] Depression and psychological disturbance may have been secondary to the extensive skin involvement, but is also a recognized part of the syndrome.[2] She experienced dramatic clearing of her skin and improvement in well-being whilst taking oral omega-3 marine triglycerides despite further progression of the tumour.

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(20) Acrodermatitis chronica atrophicans

DR C.MATTHEWS

Newport, Gwent

G.T., male, aged 55 years.

History. He gave a 2-year history of gradually increasing discoloration of the back of the left hand associated with an increasing feeling of tiredness, inability to concentrate and some vague aching in various joints. Symptoms began 1 month after he returned from living in Australia for several years.

Examination. There was dusky discoloration and atrophy of the dorsum of the left hand. There was some dryness and wrinkling of the skin of the left forearm suggestive of early morphoeic change. Neurological and cardiovascular systems were normal.

Investigations. There was a positive Borrelial antibody test (positive 1:64 on two occasions). R A Latex positive. Skin biopsy: non-specific inflammation.

Comment. Although initially reported as a positive Borrelial antibody test the laboratory subsequently felt that this was a negative result. It is known that there is variation between laboratories in determining antibody levels.[1]

The clinical picture fits very well with acrodermatitis chronica atrophicans as described by Asbrink *et al.*[2] It is interesting that changes suggestive of early morphoea were found further up the same limb, reminiscent of the case reported by Coulson.[3]

Acrodermatitis chronica atrophicans is extremely rare in the U.K. This patient had been living in Australia for 5 years immediately prior to the onset of his symptoms and in an area where there were ticks, although he had not knowingly been bitten.

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(21) 'Malignant' atrophic papulosis (Degos)

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D.T., female, aged 48 years.

History. The patient noticed painless, non-itchy spots in April 1988. They first appeared on her left thumb and subsequently on her arms, trunk and legs. She is otherwise perfectly well. In particular, she has had no symptoms indicating bowel or central nervous system disease. New skin lesions continue to appear. There was no relevant family history.

Examination. There were randomly scattered papules on her arms, legs and trunk. The papules were 3–7 mm in diameter, and most had a well-defined areola of dilated vessels with a central depressed white scarred area looking like porcelain (Fig. 1). Some lesions were red with no central scar. There were no lesions resembling vasculitis.

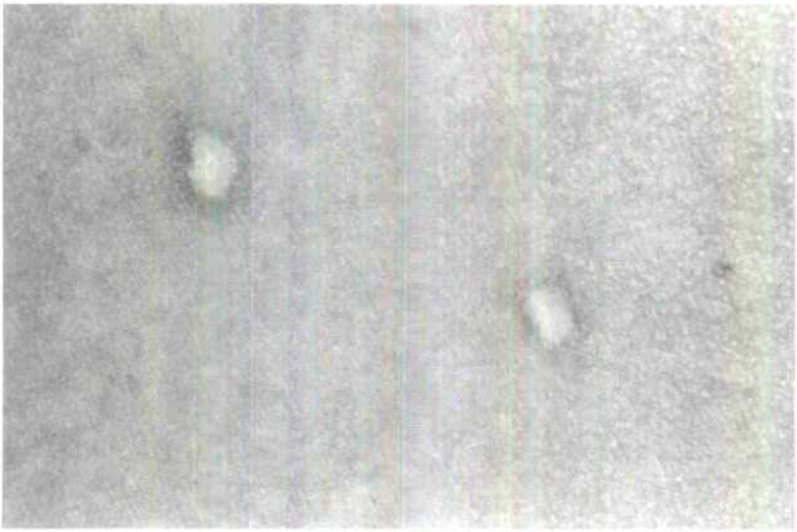

FIGURE 1. Degos' disease: typical papules with porcelain-like centre.

Investigations. Biopsy of an early skin lesion in April 1989 showed a wedge-shaped area of collagen necrobiosis with perivascular cuffing with lymphocytes at the margin. At the apex of the abnormal collagen in the deep dermis was an intense infiltrate around a small artery. There was accumulation of mucopolysaccharides in the abnormal collagen that stained with Alcian blue.

The following tests were negative or normal: biochemical screen, full blood count, autoantibody screen, C-reactive protein, C_3, C_4, platelet function tests, studies of coagulation and fibrinolysis, fibrinogen levels and occult blood × 3. IgM anti-cardiolipin antibodies were not detected, and IgG anti-cardiolipin antibodies were 2·0.

Treatment. Aspirin 75 mg daily was started in December 1989.

Comment. Degos' original patient died 10 months after the onset of the skin lesions. He then described 20 more cases, most of whom died. However, recent surveys of this disease have shown a better prognosis, and of nine cases seen at the Mayo Clinic between 1960 and 1981, six were still alive 4–14 years after diagnosis.[1] Burg and colleagues[2] reviewed 106 cases from the literature and found a mortality of 50%. Death is usually associated with involvement of the gut and central nervous system and occurs within 2–5 years of diagnosis. However, the follow-up period of many of the reported cases has been less than 5 years, which may account for this seemingly better prognosis. Recent interest has centred on abnormalities of coagulation. Cases have been reported with increased levels of fibrinogen,[3] an increased tendency to platelet aggregation[4] and impaired fibrinolytic activity of blood vessels.[5] The case described by Hughes' group[6] with high levels of anti-cardiolipin antibodies further supports the view that an increased tendency to thrombosis may be relevant in this disease. This present cases does have increased levels of IgG anti cardiolipin antibodies, although only at a low titre.

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(22) Pseudo-Degos' disease

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J.S., black male, aged 33 years.

History. Over the past 5 years, this patient has been admitted to hospital under the surgeons more than a dozen times for episodes of abdominal pain and gastrointestinal haemorrhage. At gastroscopy, sigmoidoscopy and laparotomy, haemorrhagic, ulcerative and gangrenous lesions as well as strictures have been documented. Treatment has usually been conservative but hemicolectomy was necessary in 1984.

Since the start of his illness, he has suffered with a rash distributed over the trunk and proximal limbs but absent in the interscapular area. Individual lesions arise as urticated papules which itch and burn, provoking immediate scratching and excoriation, leaving hypopigmented scars.

Examination. Multiple hypopigmented scars are present on the trunk and proximal limbs but spare the interscapular area. They do not have an adherent scale and are not porcelain-white.

Investigations. The following tests were normal: VDRL, sickle status, coagulation profile, phospholipid and autoantibodies, anti-nuclear factor and mesenteric arteriogram.

Histology. Gastrointestinal biopsies and specimens showed features of acute and chronic ischaemic changes with partial to complete obliterative changes in some arterioles. No vasculitis was observed. The skin histology showed an early eosinophil-rich infiltrate progressing to a heavy, mixed infiltrate surrounding swollen, occluded small vessels in the mid-dermis.

Comment. The association of gastrointestinal ischaemia and haemorrhage and a rash typified by discrete areas of hypopigmentation suggested the diagnosis of Degos' disease.[1] The gut histology was consistent with that diagnosis but the rash was not typical. Degos' skin lesions begin as asymptomatic papules which slowly develop central umbilication. They evolve into porcelain-white depressed scars covered with a scale and surrounded by a telangiectatic rim. This patient's lesions were extremely symptomatic, evolved quickly and were not depigmented. Sparing of the interscapular area suggested that some form of isomorphic phenomenon was operating. In addition, polymorphonuclear vasculitis was most unusual and an eosinophil-rich vasculitis has not yet been described. The hypopigmented scars showed minimal histological changes; the acellular, mucin-rich dermal infarct expected in Degos' disease was not seen. Systemic steroids have had little effect on the disease process in this patient.

Vasculitis affecting the gastrointestinal tract can be difficult to demonstrate histologically but this should have been detected in the surgical specimens.[2]

It is possible that the skin lesions could be regarded as a slightly atypical expression of Degos' disease which is rare in Blacks. The overall features suggest that a different pathological process is occurring.

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(23) Pemphigus vegetans masquerading as multiple squamous cell carcinoma

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T.C., male, aged 46 years.

History. He presented in 1988 with an 8-month history of a persistent, crusted, verrucous lesion in the right ala nasi, which was biopsied and diagnosed as a squamous cell carcinoma. A second similar lesion developed on the left side of the nose 1 month later. Both lesions were excised by the plastic surgeons and the defects closed with bilateral nasal flaps. A further similar lesion appeared on the scrotum a month later, which was also excised. The histology of all three lesions was thought to be consistent with acantholytic squamous cell carcinoma. Six months later he developed a recurrent crusted lesion in one of the nasal flaps, and a new lesion in the umbilicus. He also complained of a sore mouth and throat and hoarseness, and had extensive palatal erosions and an oedematous, inflamed epiglottis. He had been exhaustively investigated for these same symptoms 2 years earlier at the Royal National Throat, Nose and Ear Hospital, with no firm conclusion reached. An epiglottal biopsy in 1987 showed non-specific inflammation.

Investigations. Review of skin sections showed epidermal clefting and splitting, with acantholytic cells. Palatal biopsy showed similar changes to the skin biopsies. Skin direct immunofluorescence: positive, showing intraepidermal IgG with an intercellular pattern. Indirect IMF negative. Full blood count, ANF, complement normal. Ophthalmological assessment: boggy hyperaemic conjunctivae with no scarring. The clinical, histological and immunofluorescence findings are consistent with pemphigus vegetans.

Treatment. A potent topical steroid/antibiotic combination (Dermovate-NN^{TM}) produced only partial control of the skin lesions, and he is currently taking prednisolone 40 mg/day and azathioprine 100 mg/day.

Comment. Crusted verrucous lesions in intertriginous sites typically occur in pemphigus vegetans, but in this case were initially misdiagnosed as squamous cell carcinomata, both clinically and histologically. The development of recurrent skin lesions and mucosal lesions suggested the correct diagnosis, which was confirmed by immunofluorescence.

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British Society for Dermatopathology
1990, Warwick

CHAIRMAN PROFESSOR E.WILSON JONES

PROGRAMME OF MEETING

Thursday 19 July 1990: British Society for Dermatopathology

14.00	Invited speaker	
15.00	**Tea**	
15.20	**Scientific papers**	
	J.S.Ross, R.Cerio and R.D.R.Camp	The effect of autologous interleukin-1 on factor XIIIa dermal dendritic cells in normal human skin
	R.W.Groves, E.Wilson Jones, and D.M.MacDonald	Human orf and milkers' nodules: a clinico-pathological study
	N.P.Burrows and R.Russell Jones	Cutaneous necrosis in chronic renal failure
	C.B.Bunker, R.Cerio, H.A.Bull, J.Evans, J.C.Foreman and P.M.McDowd	Mast cells after capsaicin application in normal human skin
	R.K.Curley, M.E.Fallowfield, M.G.Cook and R.A.Marsden	The clinical features of dysplastic naevi: inter-observer agreement and correlation with histology
	M.E.Fallowfield and M.G.Cook	Melanocyte atypia and melanoma
	R.Cerio and E.Wilson Jones	The histogenesis of lamellar fibrosis: a new concept
	A.C.Markey, I.M.Leigh, B.Lane and D.M.MacDonald	Changes in keratin phenotype associated with malignant transformation of keratinocytes
	A.Ishida-Yamamoto, J.P.Cassella, H.Shimizu, P.E.Purkis, I.M.Leigh and R.A.J.Eady	Light and electron microscopic localization of different keratins in normal human skin using a 1-nm immunogold silver–enhanced staining method

	S.B.Bittiner, S.Variend and S.S.Bleehen	A histopathological review of 21 cases of juvenile xanthogranuloma
	A.J.G.McDonagh, D.N.Slater, D.J.Gawkrodger, P.M.Hird, C.I.Harrington and A.E.Walker	Unilesional mycosis fungoides: a review of four cases
	N.Kirkham, B.Gibson and M.L.Price	Breslow thickness of melanoma can be measured in frozen sections
17.10	**Case reports**	
	N.P.Burrows and R.Russell Jones	Cutaneous vasculitis following administration of anisoylated plasminogen streptokinase activator complex (APSAC)
	R.H.Bull, J.F.Ryan and M.G.Cook	Fatal facial ulceration
	A.Anstey, N.Beechy-Newman, L.Gowers and H.Molloy	A case of candidal umbilical granuloma
	A.J.G.McDonagh and S.S.Bleehen	Lipoid proteinosis: immunohistochemical and ultrastructural studies
	N.Nolan and S.Rogers	Sclerosing sweat duct carcinoma (microcystic adnexal carcinoma)

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BRITISH SOCIETY FOR DERMATOPATHOLOGY

SUMMARIES OF PAPERS

The effect of autologous interleukin 1 on Factor XIIIa dermal dendritic cells in normal human skin

J.S.Ross, R.Cerio* and R.D.R.Camp

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The cell marker Factor XIIIa (FXIIIa) has been shown to identify a distinct indigenous population of dermal dendritic cells (DDC) in normal human skin. Moreover, previous studies have shown a marked increase of FXIIIa$^+$ cells in atopic eczema and psoriasis. Immunophenotypic characterization suggests these cells posses mononuclear-phagocyte function and may be capable of presenting antigen, possibly under the control of soluble cytokines. We have studied the effect of epidermal-derived interleukin 1 (IL-1) on DDCs in normal adults.

IL-1α-like material was purified from stratum corneum of three volunteers by successive HPLC steps. Autologous material (60 U, quantified in an EL-4 NOB-1 cell bioassay) in 60 μl sterile phosphate buffered saline was injected intradermally in the forearm of each volunteer. Erythema and swelling developed within 2 h of injection and persisted for at least 24 h. Biopsies were performed at 4 h.

Employing a routine indirect immunoperoxidase labelling technique, Factor XIIIa$^+$ DDCs were examined in paraffin sections. FXIIIa$^+$ DDCs were present in increased numbers in IL-1 injection sites (135 ± 6 mean number of cells × 2 low powered fields ± SEM, $n = 3$) when compared with controls (100 ± 6 mean ± SEM, $n = 3$). As well as a 30% increase in the number of FXIIIa$^+$ cells following IL-1 injection, cells appeared larger and more dendritic and were located in the papillary dermis, especially around blood vessels.

This study demonstrates the response of FXIIIa$^+$ DDCs following intradermal injections of autologous IL-1. The release and action of epidermal IL-1 may therefore play a role in inducing the increased numbers of FXIIIa$^+$ DDCs documented in inflammatory dermatosis.

Human orf and milkers' nodules: a clinicopathological study

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Orf and milkers' nodule (MN) infection of the skin is a common problem in rural areas but rarely presents to the dermatopathologist. We have recently collected a series of 11 cases of nodular orf or MN from around the U.K. to reassess their clinical and histopathological features.

All patients gave a history of contact with farm animals. Most were male ranging in age from

12 to 65 years. The majority of lesions affected the hands or arms, ranged in size from 1 to 3 cm and occurred on average 3 weeks after presumed exposure (range 1–5 weeks).

On low power examination a typical architecture was present. The epidermis was papillomatous and the dermal papillae were distended by intense oedema: there was massive dermal capillary proliferation and dilatation, and an inflammatory infiltrate which variably consisted of lymphocytes, polymorphs and eosinophils. This not infrequently involved the epidermis, sometimes with intraepidermal abscess formation. High power examination revealed in all cases epidermal viral cytopathic changes with inclusion bodies, clumping of keratohyalin, and cytoplasmic vacuolation with distinctive spongiform degeneration.

We conclude that nodular orf and MN infection has a distinctive histopathology and, in contrast to some previous reports, viral changes may frequently be found.

Cutaneous necrosis in chronic renal failure

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We report three patients with cutaneous infarction in association with vascular calcification and chronic renal failure. The clinical presentation was characteristic, with livedo reticularis, subcutaneous induration and haemorrhage, affecting predominantly the proximal limbs. This was followed by widespread areas of skin necrosis and a fatal outcome in all three cases. Plain radiographs confirmed the presence of calcification of smaller arteries in relation to the affected sites. A biopsy of clinically involved, but non-ulcerated, skin showed sweat gland necrosis and red cell extravasation. More advanced lesions showed necrolytic changes affecting the epidermis, but no evidence of leucocytoclastic vasculitis or subcutaneous calcification.

The pathogenesis of this rare disorder has been compared to experimental calciphylaxis but we could find no histological support for this hypothesis.[1] The vascular calcification could be either dystrophic or metastatic.[2] Only one of our patients had hyperparathyroidism, though the other two did exhibit hyperphosphataemia. It is important to recognize that cutaneous necrosis can occur in the absence of hypercalcaemia or clotting disorders.

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Mast cells after capsaicin application in normal human skin

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Peptides released from sensory nerves during an axon reflex are thought to cause mast cell degranulation, histamine (Hi) release and Hi-induced vasodilatation leading to the flare of the triple response. Capsaicin stimulates peptide release from sensory neurones but does not cause Hi release from mast cells *in vitro*. We have now studied mast cell degranulation in human skin following the application of capsaicin.

The stratum corneum was stripped (with transparent adhesive tape) from the ventral aspect of one forearm in four normal subjects. Epicutaneous applications of 0·25 ml of normal saline (N/S) and 1% capsaicin were made to separate skin sites within this area and repeated after 1 h. Skin was biopsied (4-mm punch) from each site after 1 h and after 6 h. Specimens were examined blind by light microscopy. Mast cells in three separate (toluidine blue) fields (× 100) were counted.

There was a significant ($P < 0{\cdot}05$) reduction in the numbers of mast cells in the skin 6 h after capsaicin application compared with N/S (Table 1; mast cells/3 × 100 fields).

TABLE 1

Subject	N/S 1H	N/S 6H	Caps 1H	Caps 6H
1	24	24	21	15
2	16	17	27	11
3	18	22	25	20
4	22	19	12	12

Our data show that capsaicin treatment causes mast cell degranulation. Since capsaicin itself does not release Hi from mast cells our data suggest that capsaicin-induced release of peptides from neurones could cause mast cell degranulation. The reported failure of antihistamines to block capsaicin induced flare may imply that other mediators as well as Hi cause flare.

The clinical features of dysplastic naevi: inter-observer agreement and correlation with histology

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Accepted clinical criteria for the diagnosis of dysplastic naevi are: size greater than 5 mm, an irregular or ill-defined edge, variation in pigmentation, a macular component, and the presence of inflammation. However, many naevi which prove banal histologically display some or all of these features, whilst a significant proportion of clinically banal naevi exhibit melanocytic dysplasia histologically. The aim of this study was to measure the level of agreement between observers when evaluating pigmented lesions and to determine which clinical features best correlated with the histological diagnosis of melanocytic dysplasia. Sixty pigmented lesions were examined independently by three observers. The presence or absence of the following features, along with the clinical diagnosis, were recorded: irregular edge, ill-defined edge, irregular distribution of pigment, erythematous component, size >5 mm, and presence of a macular component. The lesions were then excised and sent in coded bottles for histopathological examination.

Agreement between observers for each attribute and for each diagnosis (based on the histology) was determined using Cohen's Kappa statistics in which the observed agreement is adjusted for that expected by chance. Agreement between raters was greatest when evaluating the size of the lesion and the presence of a macular component, and poorest for variation in pigmentation. For those lesions which showed histological melanocytic dysplasia, the commonest recorded clinical features were: variation in pigmentation, irregular edge, and a macular component. These same features were, however, recorded equally frequently in histologically banal compound naevi, suggesting poor correlation between the clinical and histological criteria for dysplastic naevi.

Melanocyte atypia and melanoma

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There is said to be a field change affecting the epidermal melanocytes adjacent to primary cutaneous melanoma, based largely upon the observations of one author.[1] We have studied a series of re-excision specimens of narrowly excised (1–2 mm margins) melanomas in order to examine this 'field change effect'. We have also studied a series of biopsies from exposed forearm skin and covered buttock skin in patients with benign and malignant melanocytic lesions and from control subjects.

Melanocyte atypia was sought, and basal melanocytes counted in 24 consecutive re-excision specimens and in the paired punch biopsy specimens from our series of 84 patients and controls. We found a minor degree of melanocyte atypia in 5/24 resection specimens, but in no case was this greater than that seen in sun-exposed skin from control patients. Melanocyte counts were approximately constant across the resection specimens and were within the ranges seen for sun-exposed skin or covered skin in our control series.

We conclude that the field change effect said to surround primary cutaneous melanomas can be explained on the basis of sun exposure.

REFERENCE

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The histogenesis of lamellar fibrosis: a new concept

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Lamellar fibrosis is a histological feature observed in many atypical melanocytic lesions but is particularly characteristic of both inherited and sporadic dysplastic naevi (DN). The aim of this study was to determine the histogenesis of the spindle cells associated with this form of architectural atypia.

Our previous studies have demonstrated that the novel cell marker Factor XIIIa (FXIIIa) detects resident dermal dendritic macrophages, especially surrounding superficial blood vessels in the dermal papillae. Most fibrocollagenous conditions such as scar and keloid are FXIIIa negative. In contrast, they are numerous in many inflammatory dermatoses.

Employing a nickel-enhanced indirect immunoperoxidase (IPO) labelling technique we examined 10 biopsies of DN. Using a chloroform-washed methylgreen counterstain, melanin produced an apple green/brown colour, nickel-enhanced IPO reaction was grey/black, and all nucleated cells stained green. Therefore, melanin and IPO could easily be distinguished.

Our findings suggest that lamellar fibrosis represents recruitment of $FXIII^+$ dermal dendritic macrophages. This results in melanophage formation and remodelling of papillary dermal collagen rather than a simple scarring process. We propose that the mechanism represents a 'walling off' of atypical melanocytes rather than a reactive fibrosis. Perhaps this process should be more accurately described as lamellar dendrocytosis.

Changes in keratin phenotype associated with malignant transformation of keratinocytes

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The keratin polypeptide profile of an epithelial cell is closely linked to the cell of origin (simple/stratified), the differentiation pathway (skin, oral, cornea) and the proliferation status of the cell. To better understand the process of malignant transformation in keratinocytes, we studied 10 cases each of *in situ* (Bowen's disease) and invasive squamous cell carcinoma (SCC) using a panel of 50 monoclonal antibodies specific to individual keratin polypeptides.

Abnormalities of keratin 14 staining were present throughout the majority of the epidermis in Bowen's disease, with delay in expression of differentiation keratins 1 and 10. SCC showed more extensive changes, with widespread expression of keratins 8 and 18, two keratins which are normally restricted to simple epithelia. Two proliferative cases of Bowen's disease also showed occasional keratin 8/18 positive cells.

Stromal invasion by keratinocytes seems to be accompanied in some cells by a switch in epithelial keratin type. Highly specific monoclonal antibodies to individual keratin polypeptides may prove to be powerful diagnostic and prognostic tools in dermatopathology.

Light and electron microscopic localization of different keratins in normal human skin using a 1-nm immunogold silver-enhanced staining method

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The localization of different keratins in normal human skin was observed using sequential light and electron microscopy. Three antikeratin monoclonal antibodies LL001, LHP1 and LP34 were used, identifying keratin 14, keratin 1 and 10 and most keratin subtypes, respectively. After fixation in paraformaldehyde-lysine periodate, the specimens were cryofixed by plunging in liquid propane at −190°C, substituted in methanol at −80°C and embedded in Lowicryl K11M at −60°C which was polymerized under UVR. Semithin and ultrathin sections were cut and incubated in the following sequence: (1) antikeratin antibodies; (2) biotinylated secondary antibody; (3) streptavidin-linked 1-nm gold particles. The gold particles were visualized by silver enhancement. Both light and electron microscopy showed that LL001 stained predominantly basal cells, LHP1 stained suprabasal cells, and LP34 stained keratinocytes throughout the epidermis. Immunolabelling was ultrastructurally restricted to keratin filaments with improved sensitivity compared with 15-nm gold labelling. The fine structure of keratin filaments, desmosomes and keratohyalin granules demonstrated good overall tissue preservation. This technique enables immunolabelling of epidermal keratins to be carried out on the same tissue blocks at the light and electron microscopic levels. The low temperature methods have the advantage of allowing good antigen expression with excellent ultrastructural preservation.

A histopathological review of 21 cases of juvenile xanthogranuloma

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Juvenile xanthogranuloma is a condition usually occurring in early childhood and characterized by small red or yellowish nodules which may grow rapidly and then usually undergo spontaneous resolution. We have reviewed the histopathological features in a series of 21 cases (eight male, 13 female).

The majority of the lesions contained many PAS-positive foam cells and Touton-type giant cells. Eighteen of the lesions involved the papillary dermis with concomitant attenuation of the epidermis. A storiform pattern of the cells was not usually a prominent feature. Van Gieson's stain showed a variable amount of collagen but little elastin. Two lesions contained large amounts of iron in the upper dermis, and one lesion had an excess number of mast cells. Stains for *Ulex europaeus* agglutinin 1 and Factor VIII-related antigen reflected the vascularity of the lesions but did not stain lesional cells. There were variable numbers of Factor XIIIa positive dermal dendritic cells contained within the lesions.

Juvenile xanthogranuloma is a distinct entity but the histogenesis of the lesions and its relationship to other histiocytic disorders in childhood remains uncertain.

Unilesional mycosis fungoides: a review of four cases

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Unilesional mycosis fungoides is very rare in clinical practice and fewer than 10 cases have previously been reported. We have recently studied four male patients with this condition. At presentation, their ages were 22, 47, 56 and 74 years and all had solitary erythematous plaques on the trunk or lower limbs.

Histological examination revealed, in each case, a dense, often band-like, lymphohistiocytic infiltrate in the dermis containing at least 95% T lymphocytes with Pautrier abscess formation in three cases. The appearances were indistinguishable from those of classical mycosis fungoides in the plaque stage. Three cases also showed poikilodermatous features; one was an apparent spongiotic variant. None had the features of Pagetoid reticulosis.

Two patients were treated by surgical excision, one by excision followed by radiotherapy, and one by topical 5-fluorouracil followed by radiotherapy. All remain well with no evidence of recurrent disease after intervals of 1–8 years.

The benign biological behaviour of these lesions is consistent with the natural history of classical mycosis fungoides. Our patients appear similar to those reported by Oliver and Winkelmann[1] but prolonged follow-up is necessary to prove such cases are truly unilesional.

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Breslow thickness of melanoma can be measured in frozen sections

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The question is sometimes asked, 'Is it possible to diagnose melanoma in frozen sections?' There are two possible answers to this. The first is that the diagnosis of melanoma should not be made rashly or precipitately. The second is that, as we have shown previously, it is perfectly possible to cut sections of melanoma, which are of high quality, and that such sections are suitable not only for morphological diagnosis but also have the advantage that a number of immunohistochemical markers can be used, which do not react in routinely fixed and processed paraffin sections. The next question is whether frozen sections are suitable for microstaging, with the implication that there may be a significant difference in the Breslow thickness of a melanoma as measured in a frozen compared to a paraffin section. We have reviewed biopsies in which the tissue was received fresh. Part was used for frozen sections and part fixed and processed routinely to paraffin wax. Breslow thicknesses were measured for each biopsy, in both preparations. A series of basal cell carcinomas was used to provide a non-melanocytic control tumour. Where paraffin and frozen sections from the same biopsy showed comparable appearances, Breslow thickness was similar to within 0·1 mm, when measured using the Vernier method. These findings show conclusively that high quality frozen sections can be used to microstage melanoma.

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BRITISH SOCIETY FOR DERMATOPATHOLOGY

CASE REPORTS

(1) Cutaneous vasculitis following administration of anisoylated plasminogen streptokinase activator complex (APSAC)

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Vasculitis has been reported as a rare complication of APSAC therapy, but histological examination has been unrewarding.[1] We have reviewed this material and report a further case with the histological features typical of leucocytoclastic vasculitis.

A 54-year-old female with non-insulin-dependent diabetes mellitus presented with an acute myocardial infarction. Five days after the administration of APSAC (30 units i/v) she developed palpable purpura on the legs and lower abdomen. Biopsy on day 6 revealed a mixed perivascular infiltrate, red cell extravasation, fibrinoid changes around dermal blood vessels and leucocytoclasia. Immunofluorescence studies in lesional skin revealed deposition of immunoreactants in a perivascular location (IgM and C3).

On clinical grounds our case fits well with the six previously reported cases. The onset was somewhat quicker (mean 10 days) but all cases cleared within 3 weeks without specific therapy. On histological grounds our case showed distinctive features of leucocytoclastic vasculitis, unlike the previous biopsies where mononuclear cell infiltration only was seen without fibrinoid change or red cell extravasation. This might reflect delay in obtaining biopsy material, but it is more likely that drug-induced vasculitis exhibits a spectrum of histological changes and is more commonly 'lymphocytic' than leucocytoclastic.[2]

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(2) Fatal facial ulceration

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Progressive facial ulceration is rare and usually secondary to malignancy. Our case represents an unusual manifestation of a rare non-malignant process which was not diagnosed before death.

A 78-year-old man presented with a large suppurating ulcer over the left zygoma. It had started as a small papule 9 months before. There was no underlying bony destruction. Recently he had been treated for recurrent 'chest infections'. Several skin biopsies were taken and these showed an intense dermal infiltrate of lymphocytes, plasma cells and histiocytes, many in the form of giant cells. No vasculitis was found. An Ethmoid sinus biopsy and bronchoscopy were non-contributory. His ESR was elevated but the anticytoplasmic antibody assay was negative. No micro-organisms were isolated on extensive culturing. He was treated for tuberculosis but died 4 months later. The post-mortem revealed congested lungs and evidence of a granulomatous vasculitis was found in both the skin and the lungs, confirming a diagnosis of Wegener's granulomatosis.

The skin is involved in up to 50% of cases of Wegener's granulomatosis but such extensive ulceration as in our case is unusual. This case stresses the need for a high level of suspicion if the diagnosis is to be made early, especially as evidence of a granulomatous vasculitis may be lacking in skin biopsies.

(3) A case of candidal umbilical granuloma

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A 14-year-old schoolboy presented with a large, protuberant mass which has arisen from red, scaly umbilical skin over 18 months. Examination revealed a 3 × 5 cm, soft, brown pigmented lesion with a finely pebbled, mildly scaly surface, protruding 3 cm above surrounding skin. There was no history of either acute inflammation or discharge from the lesion. The family history was negative. On histology there was a granulomatous inflammatory reaction within the dermis. The non-caseating granulomata were composed of epithelioid histiocytes and numerous multinucleated giant cells partially surrounded by a rim of lymphocytes. The overlying epidermis showed focal parakeratosis but was otherwise unremarkable. Ziehl-Nielson stain was negative but PAS demonstrated scanty numbers of fungal hyphae within the granulomata and occasional spores in the parakeratin. The morphological characteristics of the fungus were those of Candida and this was confirmed on culture. Treatment involved excision of the lesion followed by a 1-week course of itraconazole.

Comment. The histology from this patient is far removed from that seen in chronic mucocutaneous candidiasis where massive hyperkeratosis is more prominent than granuloma formation. Well-organized granulomas, as seen in our case, may occur in the oral mucosa but we are aware of only two cases (Professor R.Hay, personal communication).

REFERENCE

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(4) Lipoid proteinosis: immunohistochemical and ultrastructural studies

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Immunohistochemical and electron microscopic studies were carried out on biopsies taken from a 56-year-old man with characteristic yellow-brown papules on the margins of the eyelids and with involvement of the oral and pharyngeal mucosae.

There were extensive deposits in the upper dermis of PAS-positive, diastase-resistant hyaline-like material which was also around the blood vessels. Immunohistochemical staining was performed using antibodies to a number of extracellular matrix and basement membrane constituents including laminin, type III collagen, unsulphated chondroitin, chondroitin-4-sulphate, chondroitin-6-sulphate and chondroitin-4-sulphate/dermatan sulphate. The expression of these were largely normal and with no apparent staining of the hyaline-like deposits.

On electron microscopy, there were large extracellular deposits of finely granular and fibrillar material interspersed between the collagen bundles. Fine non-branching microfilaments of varying lengths were in many of these deposits. The fibroblasts had dilated cysternae of rough endoplasmic reticulum, and their cytoplasm appeared more electron dense and contained a number of vacuoles. Mast cells were frequently found and were present within hyaline-like masses. The basal lamina around the blood vessels was reduplicated and also at the epidermal–dermal junction.

Our studies show that in this rare inborn error of metabolism there are striking ultrastructural changes in the dermal fibroblasts and large amounts of finely fibrillar deposits in the dermis. However, the underlying metabolic defect still remains unknown.

(5) Sclerosing sweat duct carcinoma (microcystic adnexal carcinoma)

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A 60-year old man presented with a 4-year history of a growth on the left side of the nose. A lesion was treated at the same site 34 years earlier but, unfortunately, no information of either the mode of therapy or the histology is available. The lesion extended from the left nasolabial fold to the roof of the nose, the left cheek and the left lower eyelid. The skin was telangiectatic and indurated with a palpable ridge on the lateral border.

A biopsy was taken followed by wide excision of the area. This showed a normal epidermis beneath which there was a deeply infiltrating neoplasm growing in cords and nests within a densely sclerotic stroma. The cells were composed of keratinocytes with occasional clear cells and without evidence of mitoses. Lumina, ducts and horn cysts were present. Keratinization was abrupt without keratohyaline granules. These features were those of a sclerosing sweat duct carcinoma (microcystic adenexal carcinoma). This eccrine neoplasm should be clinically differentiated from a basal cell carcinoma and histologically from a fibrosing basal cell carcinoma, a desmoplastic trichoepithelioma and syringoma. Immunohistochemical stains for EMA and CEA confirmed ductal origin, while secretory elements were demonstrated by S-100 and Actin positivity. The keratinization suggests dual differentiation, both pilar and ductal. Alternatively, it may be consistent with derivation from the acrosyringium and hence be entirely eccrine in origin.

This slowly growing neoplasm has no metastatic potential but has a high local recurrence rate and is locally destructive. Our patient has had extensive excision and grafting and is under careful review.

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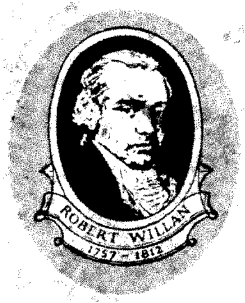

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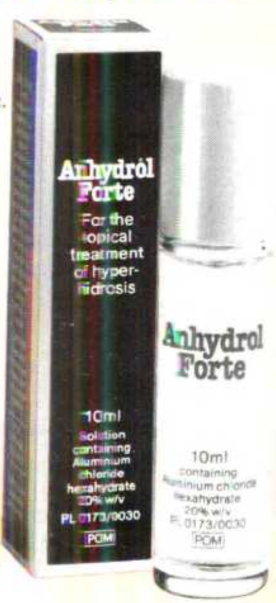
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Abnormal expression of hemidesmosome-like structures by junctional epidermolysis bullosa keratinocytes *in vitro*

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SUMMARY

Hemidesmosomes are frequently rudimentary in junctional epidermolysis bullosa (JEB), and JEB keratinocytes display abnormal attachment to the substrate in culture. Our aim was to determine whether this abnormality reflects defective hemidesmosome synthesis *in vitro*. Keratinocytes from five JEB patients were cultured under standard conditions. Control cultures, from four healthy males, three patients with dystrophic EB (DEB), and one patient with the simplex variant (EBS), were also examined. Post-confluent cultures were processed for transmission electron microscopy. Hemidesmosome-like structures were counted in electron micrograph montages. The number of hemidesmosome-like structures in JEB cultures ($0{\cdot}97 \pm 0{\cdot}57$, per 10 μm of basal cell membrane) was approximately 17% of the value for normal controls ($5{\cdot}81 \pm 3{\cdot}08$, $P < 0{\cdot}02$). The values for EBS ($3{\cdot}72$) and DEB ($3{\cdot}28 \pm 1{\cdot}44$) were not statistically different from normal controls. In addition hemidesmosome-like structures in JEB cultures were morphologically ill-defined, compared with those from controls. This correlated with the loose apposition between JEB keratinocytes and the substrate, which appeared tight in controls. JEB keratinocytes continue to express *in vitro* a major phenotypic abnormality which characterizes the disease *in vivo*. Therefore, this model should prove useful in studies determining the pathogenesis and possible new treatments of JEB.

Cultured keratinocytes from patients with junctional epidermolysis bullosa (JEB), grown under standard conditions, exhibit an abnormality of attachment to the substrate.[1,2] It was postulated that this might be due to impaired formation of hemidesmosomes *in vitro*, since this is a defect commonly associated with JEB *in vivo*,[3–6] in addition to a reduction in hemidesmosome numbers.[4–6] Cultures of JEB keratinocytes were evaluated both qualitatively and quantitatively, to determine whether these phenotypic abnormalities of hemidesmosomes were expressed

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in vitro. Control cultures of normal keratinocytes, and keratinocytes from patients with the dystrophic (DEB) and simplex (EBS) variants of epidermolysis bullosa (EB), were also examined to ensure that any changes were not a result of culture conditions.

METHODS

Subjects

Skin biopsies were taken, under local anaesthetic, from a total of nine patients with epidermolysis bullosa. In the group with JEB, three (aged 4 months–6 years) had the severe form and two (aged 20 and 59 years) had the non-lethal variant. One patient (aged 1 year) had generalized EB simplex, and three (aged 3–24 years) were affected with the mutilating recessive form of dystrophic EB. All clinical diagnoses were confirmed by electron microscopy which revealed the characteristic levels of split; low basal in EBS, within the lamina lucida in JEB, and superficial dermal cleavage in DEB.[7]

Control material was obtained from the inner thigh of four healthy males (aged 22–28 years). Suction blisters were raised at 300 mmHg below atmospheric pressure, over a 2–3 h period, and the roofs carefully dissected away.

Tissue culture

Keratinocytes were harvested by exposing isolated suction-blister roofs, or skin biopsies, to 0·25% trypsin (Gibco, Paisley, Scotland, U.K.) for 1·5 h at 37°C.[8] Cells were seeded at 10^5 cells per 35 mm collagen-coated Petri dish with a feeder layer of mitomycin-treated 3T3 fibroblasts.[8] Cultures were incubated in a standard growth medium composed of RPMI 1640 supplemented with 10% fetal calf serum (Gibco), 5 μg/ml hydrocortisone (Sigma, Poole, Dorset, U.K.), 50 ng/ml cholera toxin (Becton Dickinson, Oxford, U.K.) and a 1% penicillin/streptomycin/fungizone solution (Gibco, cat. no. 600-5240), at 37°C in a humidified 5% CO_2/air atmosphere. All cultures were fixed after attaining confluency, 20–30 days after plating.

Electron microscopy

Following the removal of growth medium, cultures were fixed *in situ* for 1 h with 2% formaldehyde and 2·5% glutaraldehyde in 0·1 M cacodylate buffer, pH 7·2, containing 5% sucrose and 0·05% calcium chloride, post-fixed with 1% osmium tetroxide for 1 h at 4°C, and dehydrated in a graded series of ethanol (30–100%). Propylene oxide was omitted from the regime since this reacted with the plastic Petri dishes. Instead, specimens were left overnight in a 50:50 ethanol:Epon mix, before being embedded in neat Epon and cured for 2 days at 60°C.[9] Areas to be examined were selected macroscopically, cut out with a hacksaw, and mounted on Epon stubs. Sections were cut perpendicular to the cell/dish interface, with the Petri dish *in situ*, in a Reichert OMU4 ultramicrotome.

Ultrathin sections, stained with 6% uranyl acetate and Reynolds lead citrate,[10] were viewed in a Jeol 100CX electron microscope. Overlapping fields of the cell–dish interface were photographed, and printed at a final magnification of approximately 50,000 ×. Precise magnifications were determined from calibration gratings photographed and printed in parallel.

Morphometry

The length of basal keratinocyte plasma membrane facing the dish was calculated with a MOP-PET semi-automatic image analysis system (Reichert–Jung, Vienna, Austria).[11] Employing the methods of Tidman and Eady,[11] montages were scored for the number of hemidesmosome-like structures on the lower surface of basal cells. A minimum length of 100 μm of cell membrane was scored per subject. Hemidesmosome-like structures were defined as either a thickened electron-dense region of the plasma membrane overlying a sub-basal dense plate (SBDP),[12] or an SBDP alone. Isolated SBDP were scored as hemidesmosome-like structures since these are the first hemidesmosome element to be synthesized *in vitro*.[13]

Although blistering occurs in cultures of both normal and EB keratinocytes,[1,14] blistered regions were excluded from measurements since they lack hemidesmosome-like structures which were presumably lost during blistering.[14]

Statistics

All numerical data were analysed using Student's *t*-test. Comparisons were made between control values from normal cultures, and those from EB cultures.

RESULTS

There was no obvious difference in the growth characteristics between normal and EB cultures and confluence was reached within 2–3 weeks. No differences were detected by inverted phase contrast light microscopy and all cultures were composed of neat polygonal cells, as reported by Leigh *et al.*[1]

In the present study, the gross ultrastructural morphology of non-blistered regions was similar in both control and EB cultures. All cultures possessed from six to nine layers of poorly differentiated cells. Cell membranes appeared ruffled, and the extracellular space widened, at all levels within the epithelium (Figs 1 and 2). Classical *in vivo* markers of differentiation, including keratohyalin granules, membrane-coating granules and peripheral envelopes were seldom

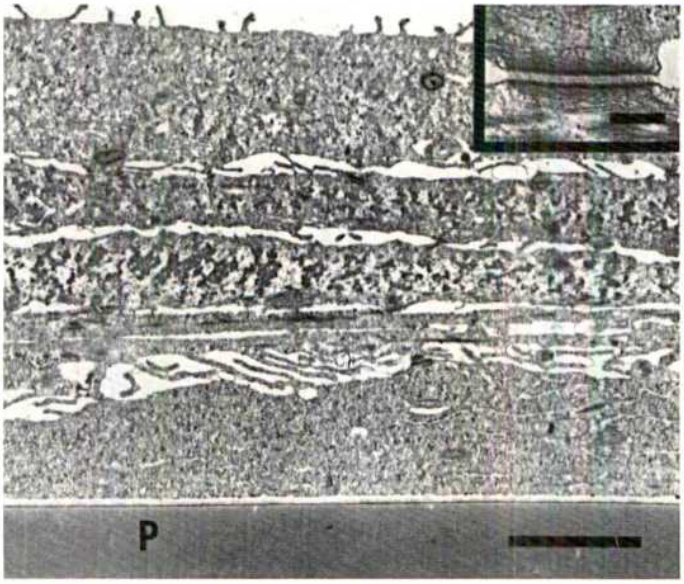

FIGURE 1. Control culture of normal keratinocytes (at 16 days). The extracellular space appears widened at all levels within the epithelium, but there is close apposition between the basal cells and the substrate. Part of the Petri dish is evident at the bottom of the micrograph (P) (Bar = 2 μm; × 8600). Insert, desmosomes retain their characteristic pentalaminar structure (Bar = 0·2 μm; × 35,500).

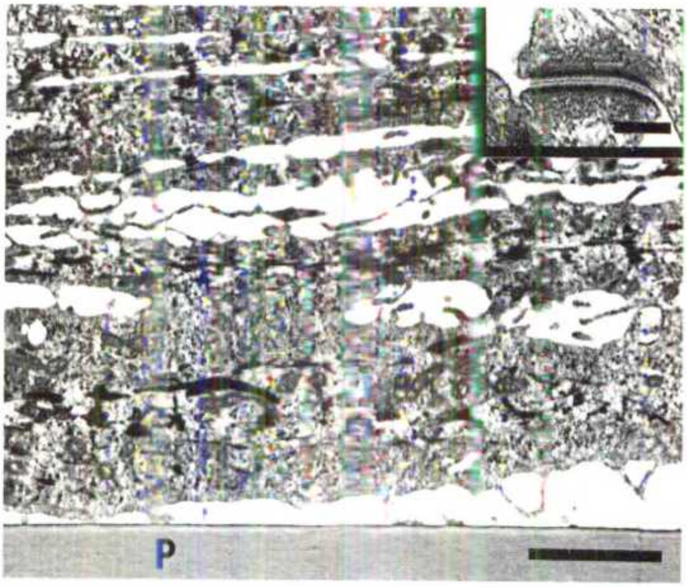

FIGURE 2. Culture of JEB keratinocytes (at 21 days) illustrating the loose apposition of the basal cells to the substrate. Part of the Petri dish is visible at the bottom of the micrograph (P) (Bar = 2 μm; × 8600). Insert, as with control cultures of normal, EBS, and DEB keratinocytes, desmosomes in JEB cultures retain their characteristic pentalaminar structure (Bar = 0·2 μm; × 35,500).

observed (Figs 1 and 2). In contrast to the stratum corneum *in vivo* which lacks intact organelles, the superficial strata of cultured epithelia frequently possessed all the cellular organelles, except the nucleus which was commonly absent in the outer 1–2 layers. Mitochondria, endoplasmic reticulum, and the Golgi apparatus were numerous in the lower epithelium, but there were fewer in the outer layers.

Desmosomes in all the cultures displayed their characteristic pentalaminar structure (Figs 1 and 2, inserts), but were greatly reduced in number compared to findings *in vivo*. Hemidesmosome morphology ranged from a single SBDP alone, to a mature hemidesmosome-like structure displaying both an SBDP and a well-defined attachment plaque (Fig. 3a). Regardless of donor type, approximately 50% of SBDP were associated with an attachment plaque, or a condensation of material resembling a rudimentary attachment plaque (Table 1). The remaining 50% presented as isolated SBDP. The percentage of hemidesmosome-like structures displaying an attachment plaque in EB cultures was not statistically different from control values (Table 1). However, there were approximately 83% less hemidesmosome-like structures in JEB cultures compared with controls, a difference which reached statistical significance (Table 1). In contrast there was no difference in the number of hemidesmosome-like structures expressed by either DEB or EBS cultures, when compared with controls (Table 1).

The reduced hemidesmosome frequency in JEB cultures coincided with what appeared morphologically to be looser attachment to the substrate (Fig. 2). In normal control (Fig. 1), EBS, and DEB cultures, basal cells were generally tightly apposed to the substrate with their cell membranes running virtually parallel to the dish surface. In contrast, basal cells in JEB cultures were frequently attached to the collagen by finger-like projections, with the main cell body separated from the substrate by extracellular dilatations (Figs 2 and 3e). Occasionally,

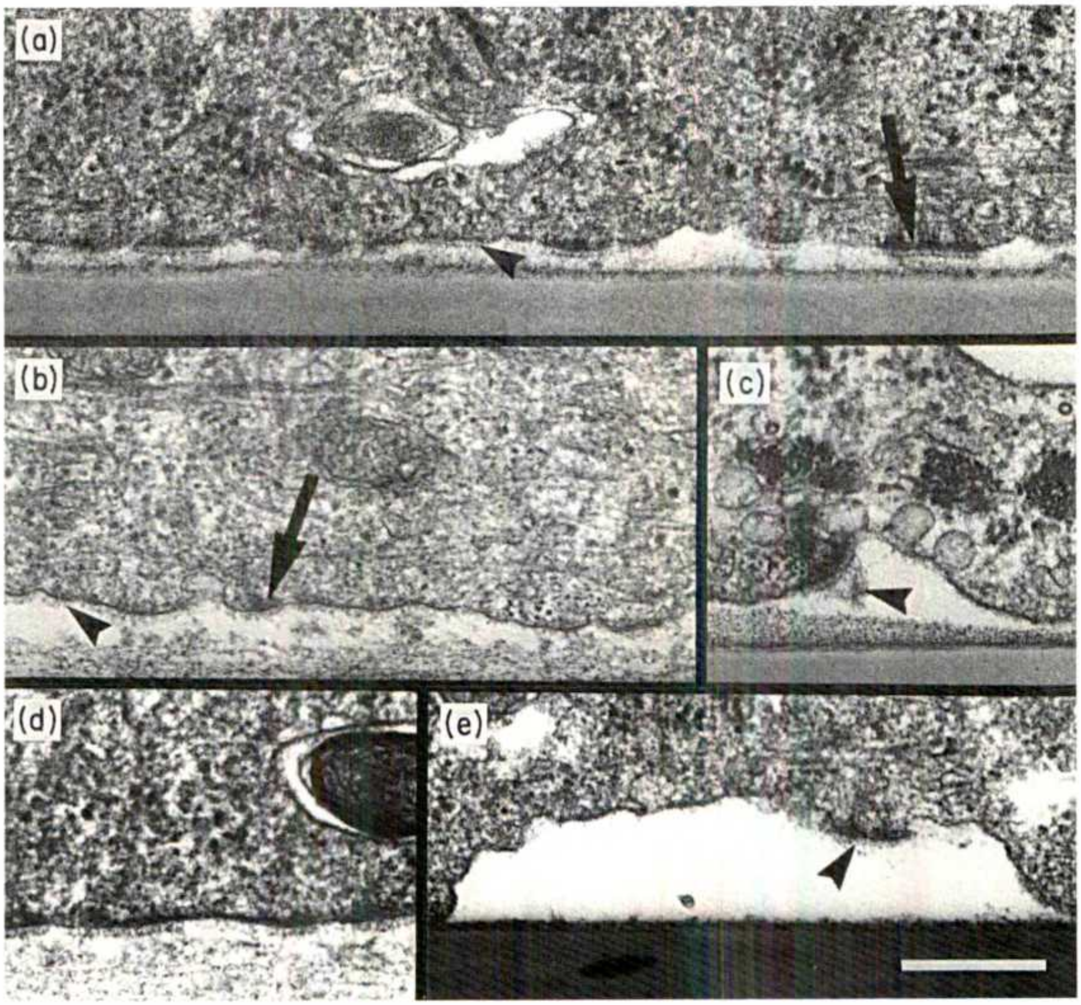

FIGURE 3. Morphology of hemidesmosome-like structures. (a) Normal control culture. The morphology of hemidesmosome-like structures ranges from a well defined attachment plaque overlying an SBDP (arrow), to structures comprising an SBDP alone (arrow head). (b) In JEB cultures the morphology of hemidesmosome-like structures is ill-defined. Attachment plaques (arrow), and SBDP (arrow head) are rudimentary. Control cultures of both EBS (c), and DEB keratinocytes (d), display the same morphological range of hemidesmosome-like structures as cultured normal keratinocytes. A small deposit of material, resembling rudimentary lamina densa, is evident beneath the hemidesmosome-like structure (c, arrow head). (e) JEB culture showing focal detachment, with a hemidesmosome-like structure (arrow head) in the roof (Bar = 0·3 μm; × 63,000).

hemidesmosome-like structures were seen on the membrane where a basal JEB cell displayed focal detachment (Fig. 3e). The hemidesmosome substructure in JEB cultures was ill-defined, exhibiting both attenuated SBDP and attachment plaques, when compared with controls or the other two EB variants (Fig. 3a–d).

A recognizable lamina densa was not detected in any culture. However, occasionally focal deposits of material resembling lamina densa were present beneath hemidesmosomes. In all

TABLE 1. Number of hemidesmosome-like structures per 10 μm of cell–dish interface (± SD)

Group	*n*	SBDP	SBDP + AP	% SBDP with AP
Control	4	5·81 ± 3·08	3·14 ± 1·39	62·63 ± 22·74
EBS	1	3·72	2·14	57·69
DEB	3	3·28 ± 1·44	1·49 ± 0·70	46·11 ± 9·96
JEB	5	0·97 ± 0·57*	0·41 ± 0·45†	37·39 ± 23·19

* $0{\cdot}02 > P > 0{\cdot}01$.

† $0{\cdot}01 > P > 0{\cdot}001$.

The number of sub-basal dense plates (SBDP) alone, and sub-basal dense plates displaying an attachment plaque (SBDP + AP) was measured, and expressed per 10 μm of cell–dish interface. The number of sub-basal dense plates associated with an attachment plaque was calculated, and given as a percentage (% SBDP + AP). Probability values (*P*) are only given where the results are statistically significantly different from control data.

cultures, in the absence of hemidesmosomes, adhesion appeared to be mediated via fuzzy, amorphous, mildly electron-dense material, possibly the precursor components of the basement membrane.

DISCUSSION

The number of hemidesmosome-like structures scored in cultures of normal, EBS and DEB keratinocytes, agreed well with results from a previous study on normal guinea-pig keratinocytes grown under similar conditions, in which 4·5 hemidesmosomes were present per 10 μm of cell–dish interface.[15] Therefore, the marked reduction in hemidesmosome numbers in JEB cultures does not appear to be an artefact. None the less, the control values for our normal, EBS, and DEB cultures (range approximately 3·5–6) were nearly a third of the value detected in normal epidermis *in vivo*[11] (18 hemidesmosomes per 10 μm of basement membrane). This indicates that our method of culturing keratinocytes does result in a lower yield of hemidesmosomes compared to findings *in vivo*.

Gipson *et al.*[16] reported that isolated sheets of rabbit corneal epithelium grafted on to the lamina densa of corneas denuded of epithelium *in vitro*, reformed the full complement of hemidesmosomes within 24 h. These authors concluded that anchoring fibrils acted as nucleation sites for hemidesmosome formation. In contrast, results from chick corneal,[17] and keratinocyte cell culture studies, including our own, indicate that hemidesmosomes are formed in the absence of anchoring fibrils.[13–15,18,19] Furthermore, recombinant studies using split skin and chorio-allantoic membrane, indicate that hemidesmosomes may induce the formation of anchoring fibrils.[12]

Tidman and Eady[5] previously demonstrated the presence of numerically and apparently structurally normal hemidesmosomes *in vivo*, in a patient with non-lethal JEB. However, we cultured keratinocytes from the same patient and noted that, as with the other types of JEB *in vitro*, the hemidesmosomes were structurally and numerically abnormal. Although this patient's hemidesmosomes appeared intact *in vivo*, they were presumably functionally abnormal, since blistering occurred in response to mild trauma, within the lamina lucida.

Clearly, an apparently morphologically intact hemidesmosome is not necessarily biochemically normal. The fact that keratinocytes from this patient synthesized abnormal hemidesmosome-like structures *in vitro* is further proof that hemidesmosome production in JEB is defective. Indeed, recent evidence indicates that two molecules potentially important in dermo-epidermal adhesion, namely GB3[20] antigen, a glycoprotein found in the lamina lucida and lamina densa beneath hemidesmosomes, and 19-DEJ-1,[21] an anchoring filament-hemidesmosome associated antigen, are absent or not expressed in the severe form of JEB. However, it is unclear whether these antigenic defects represent primary gene product abnormalities, or if they are a secondary phenomenon. For example it seems probable that basal cell migration requires the enzymatic release of cells from the basement membrane. Perhaps the primary defect in JEB involves abnormal production of enzymes and/or their regulators, and the secondary effect is the loss or reduction of specific adhesion-associated molecules. Enzyme activity *in vitro* may explain why the patient exhibiting morphologically and numerically normal hemidesmosomes *in vivo*, expressed both fewer and abnormal hemidesmosomes *in vitro*. In support of this is the observation that blister fluid from JEB patients induced a junctional split in normal skin *in vitro*, which was blocked in varying degrees by proteinase inhibitors.[22]

In epithelial sheets of rabbit cornea, the formation and maturity of hemidesmosome was found to be Ca^{2+} and calmodulin dependent. When cultures were supplemented with calmodulin inhibitors, hemidesmosomes were immature and reduced in number,[23] similar to our observations of JEB keratinocytes *in vitro*, and certain types of JEB *in vivo*.[3-6] It is interesting to speculate that abnormal formation of hemidesmosomes in JEB, both *in vivo* and *in vitro*, might be due to a defective structure or production of calmodulin.

Our observations demonstrate that JEB keratinocytes continue to express a phenotypic abnormality of hemidesmosome structure and numbers *in vitro*, in the absence of autologous dermis. Cultured JEB keratinocytes failed to express hemidesmosomes or GB3 antigen, either *in vitro*, or when grafted onto nude mice.[2] Therefore, part of the defect in JEB is due to a genetic abnormality of keratinocytes. This finding, with the observation of looser attachment in JEB cultures,[1,2] supports the suggestion that the abnormality of attachment to the substrate by JEB keratinocytes *in vitro*, is due in part to impaired hemidesmosome formation.[1,2]

Cultured JEB keratinocytes provide a potentially useful model for future investigations into the possible defects associated with JEB, such as antigen expression, enzyme production/regulation, and calmodulin synthesis. This model may also prove valuable in assessing potential therapeutic agents, such as the retinoids which promote both hemidesmosome formation[15] and GB3 antigen production[24] *in vitro*.

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α-MSH causes a small rise in cAMP but has no effect on basal or ultraviolet-stimulated melanogenesis in human melanocytes

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SUMMARY

The effects of α-melanocyte stimulating hormone (α-MSH) were studied on levels of cyclic adenosine 3′,5′-monophosphate (cAMP), melanin content and response to ultraviolet radiation (UVR) in cultured human melanocytes (HuMC). Foreskin HuMC were cultured in a hormone-supplemented system not dependent on the presence of phorbol esters. Following addition of α-MSH (10^{-6} M) there was a rise in cAMP levels maximal between 5 and 15 min to 9·4 ± 3·2 pM/10^5 cells, while control levels were 3·6 ± 0·7 pM/10^5 cells. After 7 days' culture in the presence of α-MSH (10^{-8}–10^{-6} M) the melanin content increased by only 35%, whereas Forskolin (10^{-5} M) induced a 9·5-fold rise in cAMP after 5 min and a 10·9-fold rise in melanin content after 7 days. When HuMC were irradiated daily for 6 days with UVR (Helarium fluorescent lamps emitting 20% UVB, 80% UVA) melanin content rose 2·7-fold (SE 0·3). This was unchanged or slightly reduced in the presence of α-MSH (10^{-8}–10^{-6} M). Parallel observations on Cloudman S91 melanoma cells showed that α-MSH caused only an 80% increase in melanin content after 4 days. The rise in melanin content induced by three daily UV-irradiations (2·4-fold, SE 0·5) was unchanged by α-MSH (10^{-8}–10^{-6} M). Although α-MSH induces a small rise in cAMP in HuMC this does not result in melanogenesis, and the response to UVR is not affected by α-MSH in either HuMC or S91 cells.

Ultraviolet radiation (UVR) from the sun is the major physiological stimulus for human epidermal melanocytes but the mechanisms by which UVR activates the metabolic pathways of melanogenesis are unclear. Melanogenesis can be activated in a variety of pigment cells by stimuli such as isobutyl methylxanthine (IBMX) and Forskolin that augment intracellular levels of cyclic AMP (cAMP).[1-6] It has been shown that human melanocytes undergo a 'differentiation' response involving reduced proliferation and increased melanogenesis

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following stimulation with those agents.[7] However, it has also been shown that although cultured human epidermal melanocytes are activated directly by UVR, the response is apparently mediated via cAMP-independent pathways.[8] Some other agent, therefore, may act via the cAMP-dependent pathway to activate or modulate melanogenesis and possible candidates include mediators derived from keratinocytes or systemic factors such as α-melanocyte stimulating hormone (α-MSH). Previous work has established that α-MSH can stimulate pigment cells from some species such as mice, via cAMP-dependent mechanisms.[1-6] Furthermore, Lerner and McGuire[9] claimed that α-MSH would stimulate melanogenesis in humans. However, it has since been shown that human melanocytes in culture may bind MSH, and may show a rise in cAMP levels, but this does not result in activation of tyrosinase.[2,4,10] Despite the lack of evidence for a direct effect of α-MSH on human melanocytes Bolognia and colleagues have proposed that it may play a role in UVR-activated melanogenesis.[11] Although this seemed improbable, we examined the effects of α-MSH on basal and UVR-stimulated melanogenesis as well as on levels of cAMP in cultured human melanocytes.

METHODS

Cell culture

Normal human melanocytes were obtained from foreskins (up to 7 years old). The skin was cleaned of connective tissue, cut into small pieces and incubated overnight in dispase (2 mg/ml, Boehringer, Mannheim, F.R.G.) at 4°C. The epidermis was then removed with fine forceps and disaggregated by vigorous shaking in warm trypsin (0·25%) for 10–15 min. Primary cultures were established in a culture system that uses bovine hypothalamic extract as a source of growth factors rather than phorbol esters.[12] Cell strains isolated from 10 Caucasian donors were used.

Primary cultures were maintained in Medium 199 supplemented with the following growth factors: insulin (Sigma, Poole, Dorset, U.K., 10 μg/ml), transferrin (Sigma, 10 μg/ml), tri-iodothyronine (Sigma, 10^{-9} M), hydrocortisone (Calbiochem, $1{\cdot}4 \times 10^{-9}$ M), cholera toxin (Calbiochem, 10^{-9} M) and a dialysed saline extract of bovine hypothalamus (100 μg/ml) which contains a potent growth factor for melanocytes.[12] Cells were allowed to attach for 24 h in this complete medium containing 2% fetal calf serum (FCS) after which they were maintained in serum-free medium. After approximately 3 weeks in culture any keratinocytes had degenerated and detached leaving an essentially pure growth of melanocytes with no detectable contamination by other cell types. At this time there were sufficient melanocytes for subculture.

Cloudman S91 mouse melanoma cells (Clone M-3, CCl 53.1) were purchased from Flow Laboratories and maintained in Dulbecco's modified Eagle's medium (DMEM) containing 10% FCS.

Experimental protocols

Three types of experiment were performed: (a) pharmacological stimulation in which cells were cultured continuously in the presence of agonists (α-MSH, isobutylmethylxanthine or Forskolin) added every 3 days at re-feeding; (b) observations of changes in intracellular cAMP levels following short-term exposure to α-MSH or positive control; and (c) modification of UV-induced responses by α-MSH, in which cells were exposed daily to UV-irradiation or sham treatment, and fresh medium and α-MSH added after each exposure.

Pharmacological stimulation

α-MSH (Ciba Geigy, Horsham, W. Sussex, U.K.) was used at final concentrations of

10^{-6}–10^{-8} M; Forskolin (Calbiochem) was used at 10^{-4}–10^{-6} M; isobutylmethylxanthine (IBMX, Aldrich) was dissolved in 1 drop of 1 N NaOH and diluted appropriately in phosphate-buffered saline (PBS). Human melanocytes were cultured in the presence of agonists for 9 days and S91 cells for 5 days; cultures were re-fed twice weekly. Responses were assessed by determination of cell number and melanin content.

Intracellular levels of cAMP

Cells were plated at 2×10^5 per dish in 60 mm plastic culture dishes (Falcon). They were pre-incubated in the presence of IBMX (0·8 mM) for 15 min before addition of various agonists. This is a standard manoeuvre to prevent degradation of any formed cAMP. At the selected times afterwards, the medium was aspirated and the dishes rinsed once with ice-cold PBS and then flooded with 2 ml ice-cold ethanol. The cells were scraped off with a rubber policeman, pelletted and washed once with cold ethanol. Both ethanol washes were combined and freeze dried. For assay of intracellular cAMP, 500 μl of 50 mM Tris/HCl (pH 7·4 at room temperature) containing 8 mM theophylline, 6 mM 2-mercaptoethanol and 6 mM tetrasodium EDTA was added to freeze dried cell extracts which were then assayed for cAMP by using a protein-binding assay.[13]

UV irradiation

Human melanocytes were trypsinized and seeded into dishes (35 mm diameter) at 5×10^4 cells per dish. The cells were allowed to grow for 1 week in DMEM containing 2% FCS and all the growth factors except cholera toxin. This change of medium was because M199 contains a factor(s) which inhibits UV-induced melanogenesis (Friedmann, personal observation).

Cloudman S91 cells were seeded at 8×10^4 cells per dish. Duplicate dishes were used for all experiments.

Human MC received daily UV-irradiation or sham treatment six times over an 8-day period; S91 cells received three daily irradiations. Fresh dosage of α-MSH was added daily when medium was changed. The effects of treatment were determined 48 h after the last exposure.

The irradiation source was a bank of Helarium fluorescent sunlamps (Wolff B1.01.40 W) from which approximately 80% of the spectral output is in the ultraviolet A (UVA, 320–400 nm) and about 20% in the UVB (290–320 nm).[14] These lamps do not emit biologically significant amounts of UVC. The medium was replaced with pre-warmed PBS to avoid formation of medium-derived toxic photoproducts. The culture dishes were covered with quartz lids and placed underneath the radiation source. The duration of each irradiation was 6 min; sham-irradiated cultures were treated similarly but were placed on the bench for the same length of time. Immediately after irradiation the PBS was replaced with fresh complete medium and where appropriate, α-MSH was added.

Cell proliferation and melanin content

The number of cells present per dish was determined by counting aliquots of duplicate cultures in a Coulter counter (Model ZM). For each experimental condition, the number per dish was expressed as a percentage of the number of cells present at the start of each experiment (determined by counting duplicate dishes 18 h after plating).

After removal of an aliquot for counting, the remaining cells were pelleted, the supernatant removed and the cell pellet resuspended in 100 μl 1 M NaOH. After the cells were dissolved, the mixture was diluted with 400 μl H_2O and OD_{475} determined spectrophotometrically. By comparison with a standard curve obtained using synthetic melanin (Sigma), the melanin content per cell could be obtained. Because there was considerable variation in the constitutive melanin levels of different cell lines, the results were expressed as a percentage of the untreated control. Results using this method have previously been shown to correlate well with uptake of ^{14}C-dopa, an alternative measure of melanin synthesis.[8]

RESULTS

Changes in cAMP levels

Human melanocytes. Five minutes after the addition of Forskolin intracellular cAMP levels had risen ninefold (Fig. 1). At 15 min there was no further rise. Five minutes after addition of α-MSH there was a threefold rise in cAMP levels from 3·6 (SE 0·7) pM/10^5 cells to 9·4 (SE 3·2) pM/10^5 cells (Fig. 1). There was no further increase with time up to 30 min.

S91 melanoma cells. Because much previous work has defined the changes in cAMP levels following various stimuli in S91 cells, these responses were simply used as positive controls for the methodology in two experiments. Thus, in a representative experiment, 3 min after addition of Forskolin (10^{-4} M) or α-MSH (10^{-6} M) cAMP levels rose from the basal level of 0·35 pM/10^5 cells to 9·84 pM/10^5 cells (28·1-fold) and 13·8 pM/10^5 cells (39-fold), respectively.

Pharmacological stimulation of melanogenesis

Human melanocytes. In the presence of Forskolin and IBMX, cultured human melanocytes underwent increased formation of dendritic cell processes. The cellular melanin content rose by 5–20-fold (Fig. 2) and there was a slight inhibition of cell growth (Table 1). By contrast, in the presence of α-MSH (10^{-8}–10^{-6} M) there was no change in cellular morphology or growth rate while melanin content rose by up to only 30% (Fig. 2).

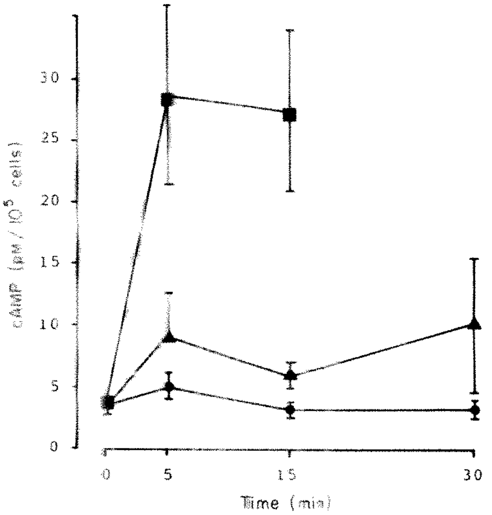

FIGURE 1. Changes in intracellular cAMP with time in human melanocytes after exposure to Forskolin and α-MSH. Duplicate dishes containing 2 × 10^5 human melanocytes were pre-incubated with IBMX (0·8 mM) to prevent degradation of cAMP. Forskolin (10^{-5} M, ■) or α-MSH (10^{-6} M ▲) were added and the reaction terminated at the times shown (see Methods). ●, unstimulated control. Points are the means and SE of four experiments.

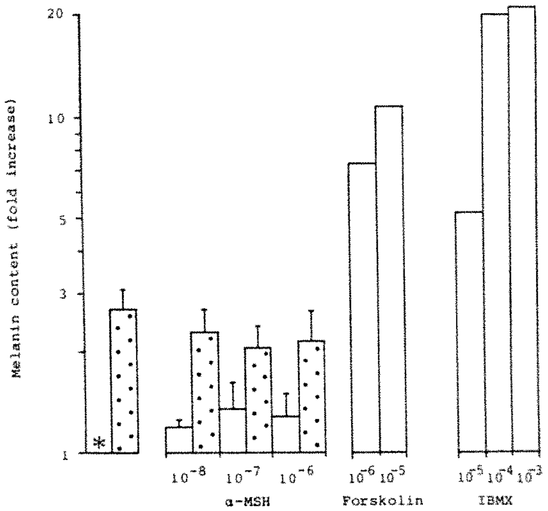

FIGURE 2. Increase in melanin content of human melanocytes after stimulation. Duplicate dishes containing 5×10^4 cells were cultured for 9 days. Controls were untreated (*) or received six daily exposures to UVB (260 mJ/cm^2, see Methods). Positive controls (one experiment only) received Forskolin or IMBX. α-MSH was added immediately after UV irradiation or sham irradiation. ▨ = UV-treated cultures. Data are means and SE (bars) from six experiments.

TABLE 1. Effects of α-MSH, Forskolin and IBMX on proliferation of melanocytes and S91 cells

	α-MSH				Forskolin			IBMX		
(Molar)	0	10^{-8}	10^{-7}	10^{-6}	10^{-6}	10^{-5}	10^{-4}	10^{-5}	10^{-4}	10^{-3}
Human melanocytes	6·8*	6·1	5·98	5·67	5·9	6·3	ND	4·6	5·8	5·8
	× / ÷ 1·01§	1·16	1·18	1·22	†	†		†	†	†
S91 cells	7·36‡	8·1	8·77	7·68	8·7	6·5	4·5	7·9	8·1	1·4
	× / ÷ 1·28§	1·26	1·23	1·28	1·7	1·6	1·4	1·8	1·6	1·4

* Data are cell numbers, geometric mean × 10^{-4}; four experiments.
† One experiment only so no error.
‡ Geometric mean × 10^{-5}; seven experiments.
§ × / ÷ geometric error.

Cloudman S91 melanoma cells. Responses to Forskolin and IBMX were similar to those seen with human melanocytes (Fig. 3). Both agents caused a dose-related increase in melanin content. Overall there was little effect upon cell growth although 10^{-3} M IBMX did produce marked growth inhibition (Table 1). α-MSH stimulated melanin content with a 2–2·3-fold rise above untreated control levels (Fig. 3).

Effects of UVR on responses to α-MSH

Human melanocytes. Six repeated exposures to an intermediate dose of UVB (260 mJ/cm^2,

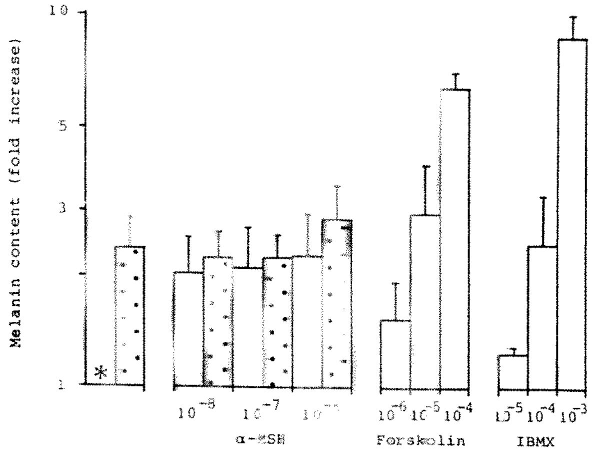

FIGURE 3. Increase in melanin content of S91 melanoma cells after stimulation. Duplicate dishes containing 8×10^4 cells were cultured for 5 days. Controls were untreated (∗) or received three daily exposures to UVB (260 mJ/cm^2 see Methods). Positive controls received Forskolin or IBMX. α-MSH was added immediately after UV irradiation or sham-irradiation. ▨, UV-treated cultures. Data are means and SE (bars) from four experiments.

unweighted for any action spectrum) caused a 2·7-fold (SE 0·3) increase in melanin content of human melanocytes (Fig. 2). In the presence of α-MSH there was no significant change in this response.

Cloudman S91 melanoma cells. Three daily exposures to UVB (260 mJ/cm^2, unweighted for any action spectrum) caused a 2·3-fold (SE 0·5) rise in melanin content (Fig. 3). This response was not significantly affected by the presence of α-MSH in the concentration range 10^{-8}–10^{-6} M.

DISCUSSION

In the present study we have confirmed the observation by Ranson *et al*[10] that α-MSH can induce an increase in cAMP levels in human melanocytes. We have also confirmed that in human melanocytes α-MSH does not stimulate melanogenesis[7] and by implication does not cause activation of tyrosinase.[4,10] Our observation that α-MSH does not modify the responses to UVR in either human melanocytes or S91 cells is novel.

Melanogenesis can be activated via cAMP-dependent pathways in both melanoma cells and human melanocytes.[1-6] In lower mammals one physiological activator of this pathway appears to be α-MSH which has been shown to induce melanization both *in vitro* and *in vivo*. There appears to be a significant difference between the responses of rodents and humans to α-MSH. Thus, human melanocytes show a rise in cAMP levels which is much smaller than that seen in S91 cells—2·5-fold compared with 39-fold, respectively. The small rise in human cells seems unable to trigger augmentation of tyrosinase activity or synthesis of melanin. This difference between mammalian species may reflect the different physiological needs of nocturnal, hair covered animals and diurnal, glabrous ones. Whether it reflects a reduced number of receptors for α-MSH on human melanocytes, a change in their affinity or a difference in other aspects of post-receptor signalling remains to be elucidated.

The finding that a α-MSH does not have a direct effect on melanin content of human melanocytes contrasts with the early claim by Lerner and McGuire[9] that α-MSH injected into humans caused an increase in melanization. However, the observed increase was predominantly in sun-exposed skin which suggested that MSH might be an indirect mediator of the effect of UVR in sunlight. Our present data show that the melanogenic response to UVB is not altered by α-MSH in either the MSH responsive S91 melanoma or MSH non-responsive human melanocytes.

It has been proposed by Bolognia *et al.*[11] that UVB might increase the activity of MSH receptors. We did not formally examine the MSH-induced changes in cAMP levels in cells that had been exposed to UVB. However, the functional consequences demonstrated that it is unlikely that there were any important changes.

In conclusion, although epidermal melanocytes of rodents and humans are activated by UVB, there seems to be a real difference between humans and rodents with regard to the responses of their pigment cells to α-MSH. If MSH plays any part in UV-induced pigmentation in humans it cannot be via a direct action on melanocytes although it might act indirectly on other epidermal cells eliciting secondary activating signals. This possibility needs to be tested before a role for α-MSH in human melanogenesis can be fully discounted.

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Epidermal growth factor receptors in human epidermal tumours

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SUMMARY

Immunolocalization studies using the strepavidin/biotin peroxidase system were used to identify epidermal growth factor receptors in 10 samples of normal skin, 13 seborrhoeic warts (SW), five lesions of Bowen's disease (BD), 11 solar keratoses (SK), 22 basal cell carcinomas (BCC) and five squamous cell carcinomas (SCC). The intensity of cell membrane staining was assessed using the 10-cm visual analogue scale technique and the results analysed using non-parametric statistics. The cell membranes in BCC showed significantly less intense staining when compared to normal epidermal cells ($P < 0.001$) and the constituent cells of SW ($P < 0.001$), SK ($P < 0.001$), BD ($P < 0.01$), and SCC ($P < 0.001$). Classification of the specimens into normal, benign, premalignant and malignant indicated that cell membranes of malignant tumours showed significantly less staining than the other groups ($P < 0.001$). The observed reduction in the epidermal growth factor receptors in malignant tumours may be due to occupation and down regulation in response to autocrine secretion of growth factors.

Epidermal growth factor (EGF), first described in 1962,[1] is a small polypeptide of 53 amino acid residues and has a molecular weight of 6045 Da. EGF acts through specific receptors present on the cell membrane.[2] The receptor is a 170 kDa transmembrane protein consisting of an external part which binds to EGF and an intracellular part which possesses the tyrosine kinase activity.[3] The binding of the ligand to the receptor induces receptor clustering, internalization of receptors, activation of receptor kinase activity and various cytoplasmic events within the cell, ultimately leading to cell division.[3] In normal human epidermis EGF receptors are present mostly in the basal and suprabasal layers rather than in other layers of the epidermis.[4] EGF receptors are also present in many other human tissues but their physiological role is not yet understood.

Several reports have demonstrated that EGF receptor levels are increased in human tumours.[5–7] Whether this increase is a marker of malignancy or signifies an important step in the development of neoplasia is poorly understood. A few studies have attempted to correlate the

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degree of receptor expression with the malignant potential of the tumour. In human bladder tumours the more invasive the tumour, the higher the EGF receptor expression,[8] and metastatic breast tumours have higher EGF receptor expression than the parent tumours.[9] Preliminary biochemical studies on human epidermal tumours have suggested some changes in EGF receptor expression.[10]

The aim of this study was to examine by immunolocalization techniques the distribution and expression of EGF receptors in epidermal tumours of varying malignancy using a well characterized monoclonal antibody to EGF receptor, designated as EGFR-1.[11]

METHODS

Materials

A monoclonal antibody to EGF receptor (EGFR-1) and the strepavidin/biotin peroxidase system were obtained from Amersham (Little Chalfont, Bucks, U.K.). Anti-mouse IgG was obtained from Sigma (Poole, Dorset, U.K.). Other agents used were of analytical grade.

Patients

Specimens were collected from patients attending the University Hospital of Wales. A total of 66 specimens were collected (Table 1). The study received approval from the Joint Ethics Committee of the South Glamorgan Area Health Authority/University of Wales College of Medicine.

Sectioning of the specimens

All specimens collected were immediately embedded in OCT compound (Chance Propper) and snap frozen in hexane at −70°C and stored at −190°C. Specimens were sectioned at 7 μm and placed on slides treated with 0·1% poly-l-lysine. Each specimen was assessed as follows: one section was stained with haematoxylin and eosin after brief fixation to confirm the diagnosis, one section was used as a control for 'indirect' immunoperoxidase staining and one section for the immunoperoxidase test itself.

TABLE 1. Details of age and sex of patients in the study

Lesions	Patients Age (years)	 Sex	Number of specimens
Normal skin	20–74	3 F 7 M	10
Seborrhoeic warts	36–83	10 F 3 M	13
Solar keratoses	55–78	4 F 7 M	11
Bowen's disease	60–70	2 F 3 M	5
Basal cell carcinomas	50–95	12 F 10 M	22
Squamous cell carcinomas	45–88	2 F 3 M	5

Immunohistochemical staining

EGF receptors were detected by the strepavidin/biotin peroxidase system (Amersham). Controls consisting of normal skin were always run in parallel with the tumour specimens. Cryostat sections were air-dried and fixed in acetone:chloroform (1:1) for 5 min at 4°C. Sections were then treated with 0·3% hydrogen peroxide (H_2O_2) to block the endogenous peroxidase for 30 min, followed by 1% bovine serum albumin and 1% normal goat serum to block any non-specific binding. Subsequently the sections were incubated with the antibody (dilution 1:100), followed by anti-mouse IgG biotin labelled and strepavidin horseradish peroxidase complex at 37°C in humid chambers. Sections were washed with Coon's buffer and the receptor visualized using DAB and H_2O_2.

Assessment

The staining intensity was assessed using visual analogue scales (VAS). The VAS technique enables a parameter which is difficult to quantify to be assigned a value by marking a 10-cm line depending on the observer's subjective assessment of the degree of change present.[12] On the VAS scales used, '0' indicated the absence of staining and '10' indicated a 'maximum' deposition of reaction product. Comparisons were made between the basal layers of the tumours and the basal layers of normal epidermis. The score for the normal epidermis was arbitrarily assessed at 5, so that it would be possible to detect a staining intensity of less than the normal. Each batch of slides contained normal controls which scored 4·96 ± 0·4 against the arbitrary value of 5 giving assurance of uniformity of the technique used. Two independent observers were used and there was a good correlation between them (using Spearman's rank correlation coefficient, $P < 0{\cdot}01$).

RESULTS

Distribution of EGF receptors in normal epidermis and epidermal tumours

In normal epidermis EGFR-1 showed a higher pericellular staining intensity in the basal and suprabasal layers than in other layers of the epidermis. As differentiation occurred from the basal layer to the stratum corneum, the staining intensity decreased uniformly. The staining pattern of various layers in the epidermis with EGFR-1 and the absence of staining with the control anti-mouse IgG is shown in Figure 1.

In tumours, differing characteristic distributions of EGF receptors were seen. All seborrhoeic warts showed staining intensities in the basal layer higher than, or similar to the differentiated compartment, but staining intensity decreased as the constituent cells differentiated above the basal layer (Fig. 2). In Bowen's disease and solar keratoses, the basal epidermal layers showed sometimes higher and sometimes lower staining intensities than the differentiated compartment with either a focal or a uniform distribution. Within the differentiated compartment itself the staining intensity of individual tumour cells varied. In SCC, a similar picture was evident in the basal layer. Within the tumour itself the staining among the tumour cells also varied (Fig. 3). In BCC, all tumours showed a uniform marked decrease in staining (Fig. 3). In some specimens virtually no staining was apparent.

VAS staining intensity assessment

For most types of tumours the staining intensity relative to the normal epidermis varied, with some specimens showing increased staining and others showing decreased staining. Consistent results were seen only with BCC, as all these lesions showed a marked decrease in staining relative to the normal epidermis.

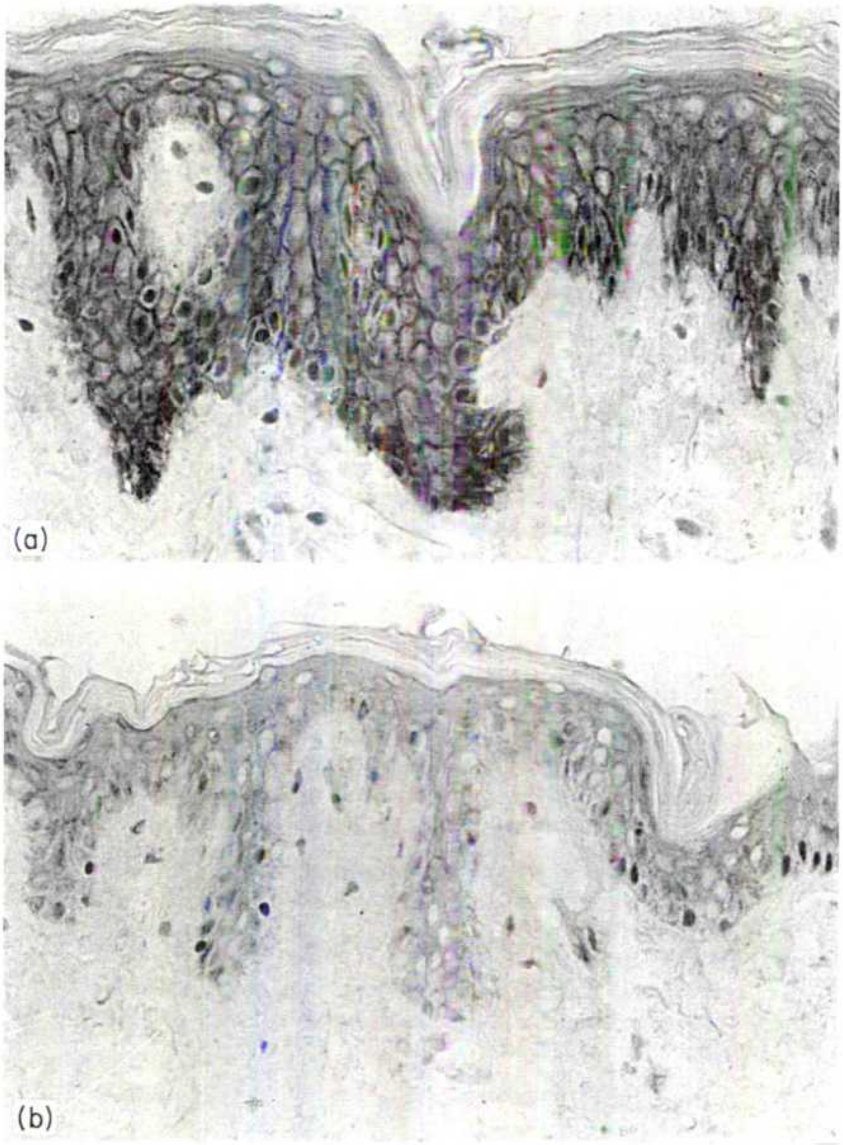

FIGURE 1. Sections of normal skin reacted with (a) EGF receptor antibody EGFR-1 and (b) mouse IgG, sections counterstained with haematoxylin. Positive pericellular staining is seen with a higher intensity in the basal and suprabasal layers.

The VAS scores for individual tumours are given in Figure 4, with the mean VAS scores and summary of statistical analysis using a non-parametric statistical analysis (Kruskall–Wallis analysis of variance) followed by multiple comparisons using a Mann–Whitney *U*-test. Values for BCC were significantly different from all other tumour types at $P < 0{\cdot}01$ or $P < 0{\cdot}001$.

Similar statistical analyses were used for comparison between normal, benign (SW), premalignant (BD, SK) and malignant tumours (BCC, SCC). The malignant group was found to be statistically different from other groups at $P < 0{\cdot}001$. (Fig. 5).

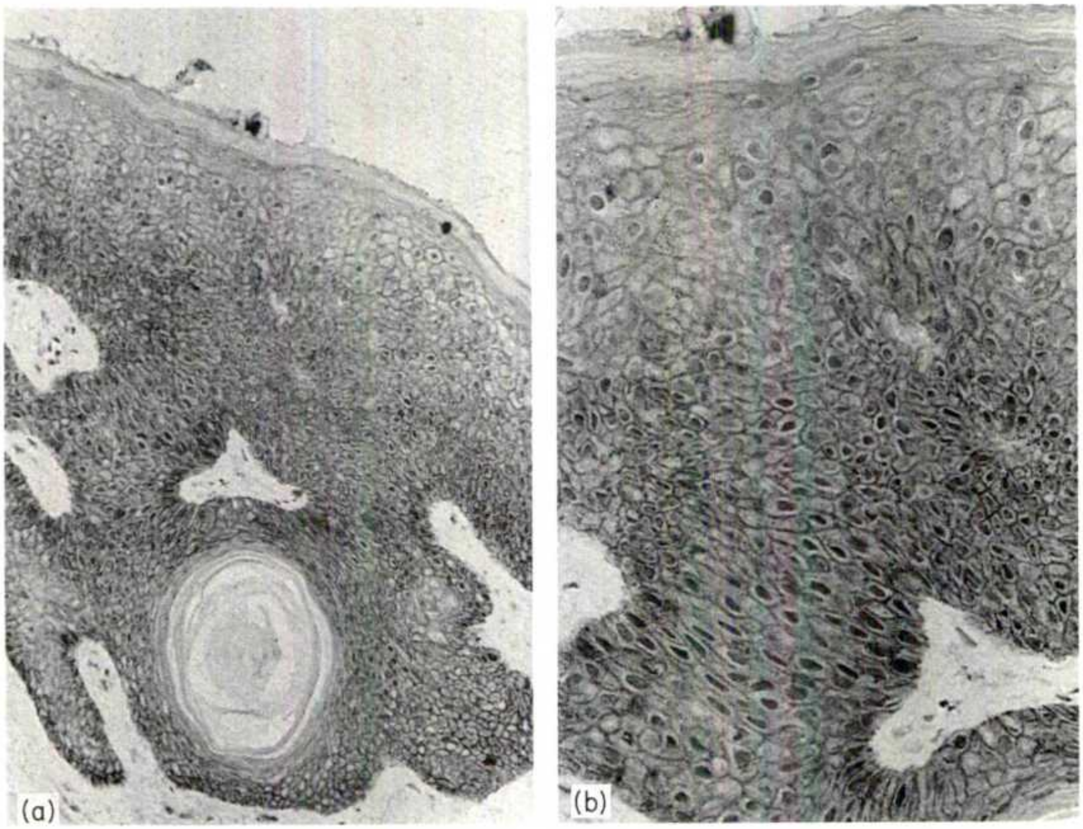

FIGURE 2. (a) Sections of seborrhoeic wart reacted with EGFR-1 showing increased staining at the base and uniform differentiation. (b) Higher magnification.

DISCUSSION

The results of this study show that in normal epidermis, the immunocytochemical reaction for EGFR-1 was greater in the basal and suprabasal layers. As the basal cell differentiates to form stratum corneum the staining intensity decreases uniformly, indicating a similar decrease in EGF receptors. This result agrees with previous studies using autoradiographic localization[4] and immunolocalization[13] for EGF receptors in normal epidermis.

In seborrhoeic warts, the staining pattern was similar to that in normal skin, with the basal layer showing higher staining intensity than the other layers. As the cells became differentiated, moving towards the surface, the staining decreased uniformly, as in normal skin. This may indicate that the differentiation sequence in these lesions is preserved, although the points at which the various events occur differs.

In solar keratoses, Bowen's disease and SCC, the pattern of distribution of EGFR-1 was entirely different. Within the tumour cells the staining intensity varied enormously. This may suggest that the level of EGF receptor relates to the cell's dysplastic state in a given tumour. All tumours showed positive staining except for BCC where no staining of tumour cells was seen.

No detailed studies of EGF receptor expression have been reported for benign and

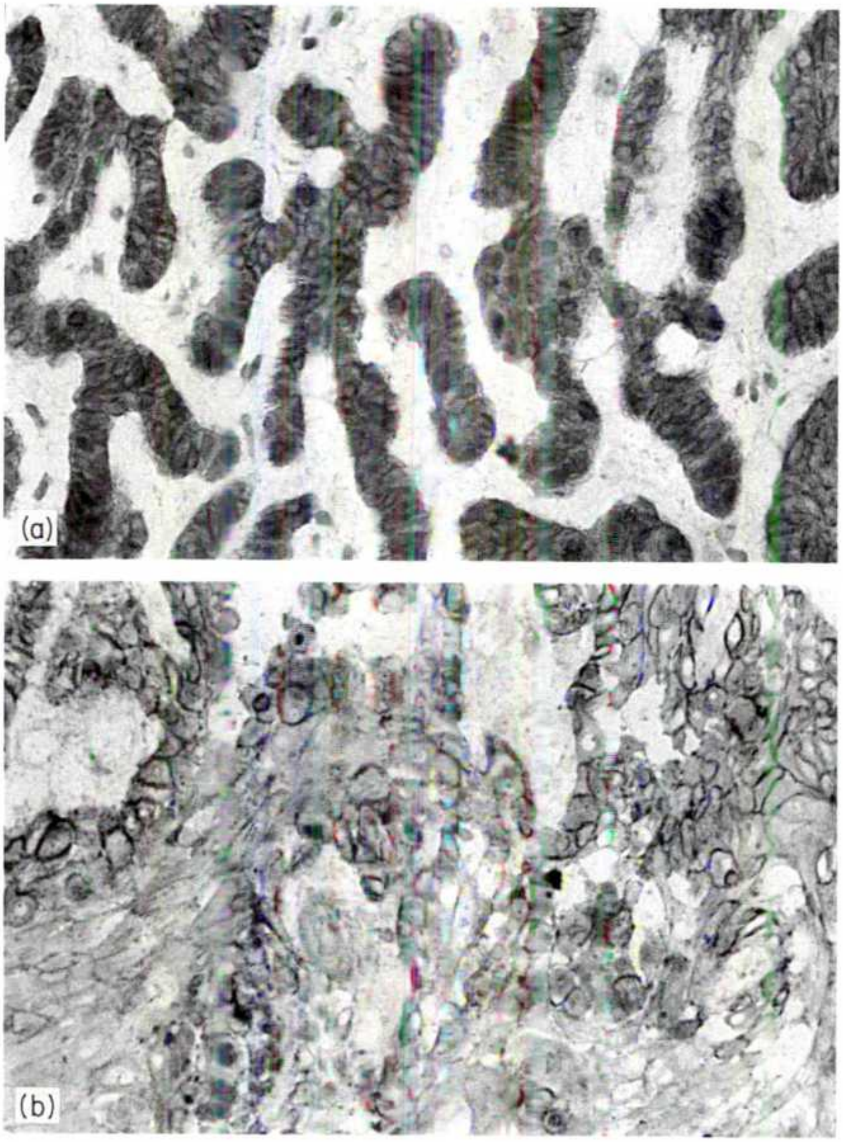

FIGURE 3. Sections of SCC and BCC reacted with EGFR-1. (a) BCC showing decreased staining with lack of staining of tumour cells. (b) SCC showing higher staining intensity at the periphery of tumour islands. Note the difference in staining intensity within the tumour cells.

premalignant tumours of the skin. Bauknecht *et al.*[10] studied a wide range of epidermal tumours but the numbers in each diagnostic category (there were 15 separate types of skin tumours examined) were too small to show whether there was any relationship between receptor expression and the degree of malignancy. However, the highest binding capacities were seen in SCC. In this study seborrhoeic warts, Bowen's disease, solar keratoses and SCC showed similar staining intensities (although a different distribution) to normal. In contrast, BCC showed a

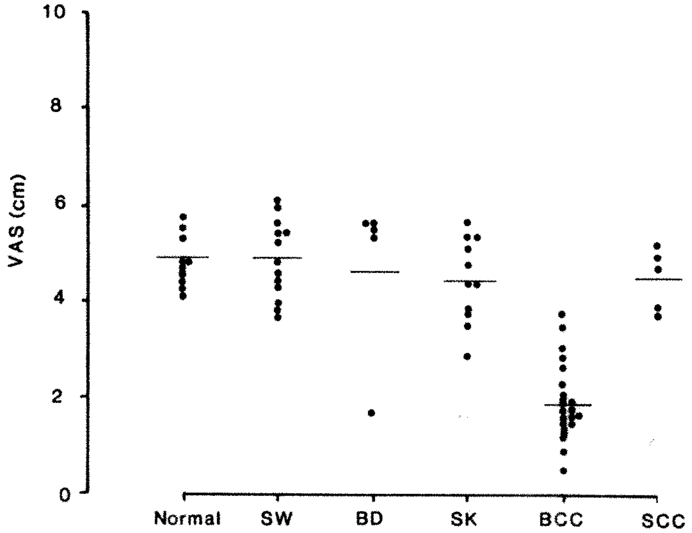

FIGURE 4. The results of the VAS scores on 10 normal skin specimens (mean = 4·96), 13 seborrhoeic warts (mean = 4·87), five Bowen's disease (mean = 4·72), 11 solar keratoses (mean = 4·87), 22 basal cell carcinomas (mean = 1·88) and five squamous cell carcinomas (mean = 4·62). The staining intensities of the basal layers of the epidermis were compared to basal layers of the tumoural epidermis. A non parametric statistical analysis by Kruskall–Wallis analysis of variance showed $P < 0{\cdot}001$ ($H = 41{\cdot}99$) and multiple comparisons with Mann–Whitney *U*-tests indicated that BCC were significantly decreased in staining intensity relative to SW, SK, BD and SCC at $P < 0{\cdot}01$ or $P < 0{\cdot}001$.

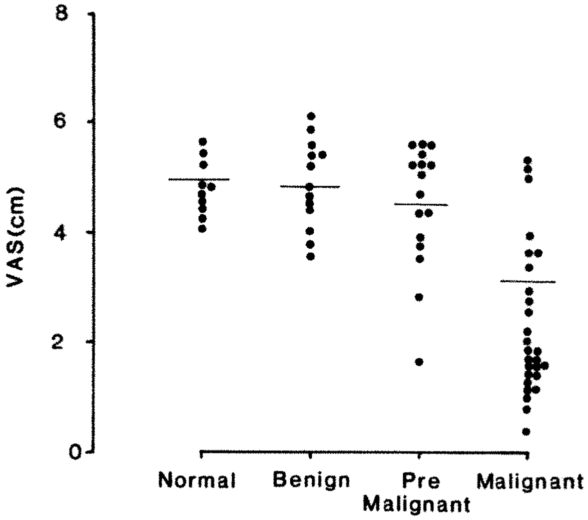

FIGURE 5. The VAS scores dividing the tumours into normal, benign (SW), premalignant (BD, SK) and malignant (BCC, SCC) groups. A non-parametric statistical analysis using Kruskall–Wallis analysis of variance showed $P < 0{\cdot}001$ ($H = 31{\cdot}87$). Multiple comparisons by the Mann–Whitney *U*-test indicated the malignant group is significantly decreased relative to normal, benign and premalignant groups at $P < 0{\cdot}001$.

significantly decreased staining intensity. This result is similar to other studies using the same antibody, EGFR-1.[14]

The reduction in EGF receptor expression in BCC may have several explanations. The EGF receptor may be so disrupted that it is beyond recognition by the antibody EGFR-1. This is quite unlikely as Bauknecht *et al.*[10] using ^{125}IEGF, showed that six out of 18 BCC bound EGF specifically. This in itself indicates that expression of EGF receptors in BCC is low and that functional EGF receptors are present on cell membranes of BCC which should be able to be recognized by EGFR-1. Bauknecht *et al.*[10] also showed that 12 BCC had negative binding to ^{125}IEGF which they suggested was due to endogenous masking of EGF receptors by tumour-derived growth factors. EGF and TGFα have been shown to be present in gastric tumours[15] and BCC,[16] respectively. This is consistent with the autocrine hypothesis in which tumours can secrete their own growth factors.[17] TGFα and EGF are related molecules and it is possible that in BCC the receptors may be occupied by either of these growth factors. Alternatively, having occupied the receptors, a down regulation of the receptors may occur which results in decreased EGF receptor expression.

To address this question in more detail, biochemical assessment of the EGF receptor content is required or *in situ* hybridization of EGF receptor using specific probes. However, EGFR-1 has been shown to recognize EGF occupied receptors[11] and thus the reduction in EGF receptor expression in BCC seems unlikely to be due to occupation of receptor sites by EGF although it may be occupied by other growth factors. To verify this, studies are being conducted to assess the presence of EGF or TGFα in human epidermal tumours of varying malignancy in relation to EGF receptor expression using EGFR-1.

Dividing the tumours into groups showed that the malignant group, BCC, had a significantly decreased staining intensity, whereas the VAS values for SCC were similar to the VAS values in normal skin. It is possible that in human epidermal tumours the expression of EGF receptors is inversely related to the presence of EGF or TGFα within the tissues.

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Radioligand binding characteristics of β_2-adrenoceptors of cultured melanoma cells

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SUMMARY

The existence of β-adrenoceptors on intact cells of the human malignant melanoma cell line A-375 was investigated using the binding properties of the tritiated radioligand (−)-[^{3}H]CGP-12177, a hydrophilic non-selective β-adrenoceptor antagonist. Displacement experiments of the radioligand from its binding site were performed with antagonists and agonists to determine the β-adrenoceptor subtype selectivity. The binding of (−)-[^{3}H]CGP-12177 was saturable, of high affinity ($K_D = 0{\cdot}025$ nmol/l, $n = 12$) and was rapid and readily reversible. The maximal number of binding sites (B_{max}) was $33{\cdot}5 \pm 1{\cdot}9$ fmol/10^7 cells or 2018 ± 114 receptors per cell. β-adrenoceptor antagonists inhibited binding of the radioligand with monophasic displacement curves. IC_{50} values were (nmol/l): propranolol (non-selective) 2·82, alprenolol (non-selective) 2·0, ICI 118,551 (β_2-selective) 3·5 and bisoprolol (β_1-selective) 2200. Agonists inhibited binding in the order of potency of isoprenaline > adrenaline > noradrenaline. It is concluded that cells of the melanoma cell line A-375 contain a homogeneous population of β_2-adrenoceptors.

Catecholamines act on target cells through stimulation of α- and β-adrenoceptors.[1] Functional studies and radioligand binding experiments have classified these adrenoceptors with specific agonists and antagonists[2–4] and their existence in almost all human tissue is certain. However, there is no information on adrenoceptors of human pigment cells. There have been studies on β-adrenoceptors in the B16 murine melanoma cell line[5] and on the melanophores of fish and amphibia.[6–9] These studies have indicated that the aggregation and dispersal of melanosomes within melanophores is mediated, respectively, via α- and β-adrenoceptors. We have investigated whether the rapidly growing malignant human melanoma cell line A-375 expressed β-adrenoceptors on their cell membranes. We also wished to classify the subtype selectivity of

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these β-adrenoceptors and have examined the potency of such agonists as isoprenaline, adrenaline and noradrenaline.

METHODS

Cell culture

The experiments were carried out using the human melanoma cell line A-375.[10–13] Monolayer cultures were grown in plastic culture flasks under 5% CO$_2$ and 95% air at 37°C and with Dulbecco's minimal essential medium (DMEM, Gibco, Grand Island, NY, U.S.A.) and supplemented with 10% fetal calf serum. The same batch of serum was used for all cultures and experiments were performed on cells from a single line of A-375 cells. The cells were cultured for 3–4 days until confluence was reached, and then after removal of the medium cells were separated with 0·025% trypsin and 0·01% versene in phosphate-buffered saline. The cell suspensions were then centrifuged at 800 ***g*** for 2 min, and the pellet resuspended in DMEM and either subcultured into two new culture flasks or used for the binding assays. The number of cells was counted before the experiments.

Binding assays

The radioligand used for binding assays was $(-)$-[^{3}H]CGP-12177(($-$)-4-(3-t-butylamino-2-hydroxypropoxy)- [5,7-^{3}H] benzimidazol-2-on), a non-selective β-adrenoceptor antagonist.[14,15] Due to its hydrophilicity this radioligand does not penetrate into cells and can be considered appropriate for characterizing membrane receptors in intact cells.[14,15] Assays were carried out in duplicate. Aliquots of 200 μl cell suspension (5×10^6 cells/ml) were incubated with 100 μl $(-)$-[^{3}H]CGP-12177 (concentration range in assays: 0·025–8·0 nmol/l) and 100 μl DMEM. The time of incubation was 90 min and the temperature was kept constant at 37°C. These conditions allowed complete equilibration of the radioligand with the binding sites. Incubation was terminated by rapid vacuum filtration (Cell Harvester M-12R, Brandel, U.S.A.) through Whatman GF/C filters and two 5 ml washes with ice-cold buffer (50 mmol/l Tris-HCl, 10 mmol/l MgCl$_2$, pH 7·4). The filters were dried at room temperature, 5 ml scintillation fluid added (Biofluor, NEN) and radioactivity was determined by liquid scintillation spectrometry in a Beckman LS 3800 counter. Specific binding was calculated by subtracting non-specific binding (in the presence of 5 μmol/l propanolol) from total binding. The maximal density (B$_{max}$) and apparent affinity (K_D) of binding sites were obtained in individual experiments from Scatchard plots determined by linear regression analysis as previously described.[16]

Kinetics

The time courses of the association and dissociation from binding sites on A-375 cells by $(-)$-[^{3}H]CGP-12177 were determined. The radioligand concentration was 0·5 nmol/l. After association reached a steady state, dissociation was started by the addition of a high concentration (100 μmol/l) of the non-selective β-adrenoceptor antagonist alprenolol, to displace the bound radioligand in a time-dependent way. From the time courses of the $(-)$-[^{3}H]CGP-12177 association and dissociation the association rate constant (k_{on}) and the dissociation rate constant (k_{off}) were calculated as previously described.[17] Association was measured during a time course of 120 min and dissociation up to 90 min. According to the mass law of action, the equilibrium dissociation constant (K_D) was obtained as a ratio of the binding reaction's reverse and forward rate constants ($K_D = k_{off}/k_{on}$).

Antagonist and agonist displacement

In antagonist and agonist displacement experiments, 1 nmol/l (−)-[^{3}H]CGP-12177 was displaced by the non-selective antagonists propranolol and alprenolol, the β_1-selective antagonist bisoprolol,[18,19] the β_2-selective antagonist ICI 118,551 (erythro-(+/−)-1-(7-methylindan-4-yloxy)-3-isopropylaminobutan-2-ol),[20] and the agonists isoprenaline, adrenaline and noradrenaline. Displacement experiments were carried out in a concentration range 10^{-10}–10^{-4} mol/l for antagonists and 10^{-7}–10^{-4} mol/l for agonists. IC_{50}-values (inhibitory concentrations) presented are the antagonist or agonist concentrations that displace 50% of the bound radioligand from the binding site.

Drugs

The drugs used were from the following sources: (−)-alprenolol-d-tartrate, (−)-isoprenaline hydrochloride, (−)-adrenaline bitartrate and (−)-noradrenaline bitartrate (Sigma, Deisenhofen, F.R.G.); (+/−)-propranolol hydrochloride (Rhein-Pharma, Heidelberg, F.R.G.); (+/−)-bisoprolol hemifumarate (Merck, Darmstadt, F.R.G.); (+/−)-ICI 118,551 (ICI-Pharma, Plankstadt, F.R.G.); (−)-[^{3}H]CGP-12177 (specific activity 53·1 Ci/mmol, Amersham Buchler, Braunschweig, F.R.G.). All other materials used were of the best grade commerically available.

Statistics

The values presented are means ± SEM.

RESULTS

β-adrenoceptor binding

Scatchard analysis of the saturation curves of all experiments ($n = 12$) revealed a single component, high affinity binding of (−)-[^{3}H]CGP-12177 on A-375 cells. Non-specific binding at the K_D-value averaged 12% of total binding. Figure 1 shows the concentration-dependent (0·025–8·0 nmol/l) (−)-[^{3}H]CGP-12177 binding to β-adrenoceptors. B_{max} and K_D, calculated from linearized binding plots of each individual experiment, were 33·5 ± 1·9 fmol/10^7 cells and 0·25 ± 0·02 nmol/l, respectively (Fig. 2). The B_{max} value corresponds to 2018 ± 114 receptors on a single cell.

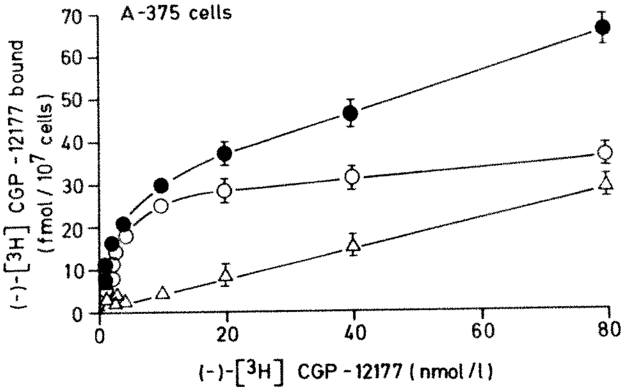

FIGURE 1. Total, specific and non-specific (−)-[^{3}H]CGP-12177 binding to intact A-375 cells as a function of increasing radioligand concentrations. Binding was determined at nine concentrations of (−)-[^{3}H]CGP-12177 ranging from 0·025 to 8·0 nmol/l. Data represent mean values ± SEM of 12 saturation experiments. ●, Total bound; ○, specific bound; △, non-specific bound.

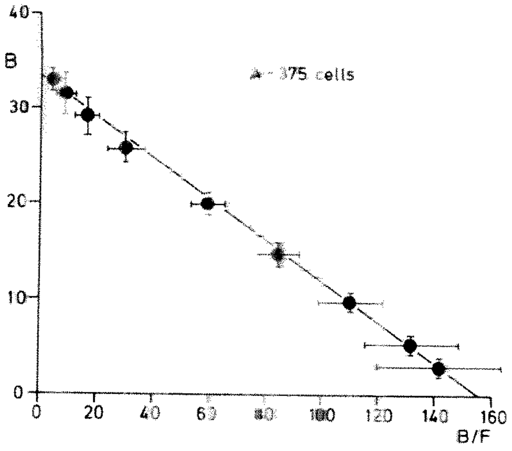

FIGURE 2. Linearized binding plot of specific (−)-[^{3}H]CGP-12177 binding to β-adrenoceptors of A-375 cells. Data represent mean values ± SEM of 12 saturation experiments. (−)-[^{3}H]CGP-12177 bound in fmol/10^7 cells (B) was plotted as a function of the ratio (B/F) of bound (−)-[^{3}H]CGP-12177 in fmol/10^7 cells to free (−)-[^{3}H]CGP-12177 in nmol/l. The intercept with the ordinate (33·5 ± 1·9) represents the maximal number of binding sites in fmol/10^7 cells (B_{max}), the slope (0·25 ± 0·02) represents the equilibrium dissociation constant in nmol/l (K_D).

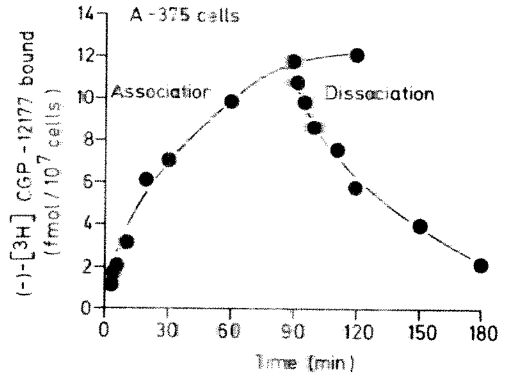

FIGURE 3. Time courses of (−)-[^{3}H]CGP-12177 (0·5 nmol/l) association with and dissociation from β-adrenoceptors on A-375 cells. Each value is the mean of a single experiment performed in quadruplicate. The equilibrium dissociation constant (K_D) from this experiment (0·67 nmol/l) was calculated as the ratio of dissociation rate constant (k_{off}) and association rate constant (k_{on}).

Kinetics

Association of (−)-[^{3}H]CGP-12177 with and dissociation of (−)-[^{3}H]CGP-12177 from β-adrenoceptors on A-375 cells are shown in Figure 3. The K_D value calculated from this experiment as a result of k_{off}/k_{on} ($k_{off} = 2{\cdot}856 \times 10^{-4}$ l/s, $k_{on} = 424151$ l/mol s) is 0·67 nmol/l. This value was found to be in reasonable agreement with the K_D value derived from the saturation experiments (0·25 nmol/l).

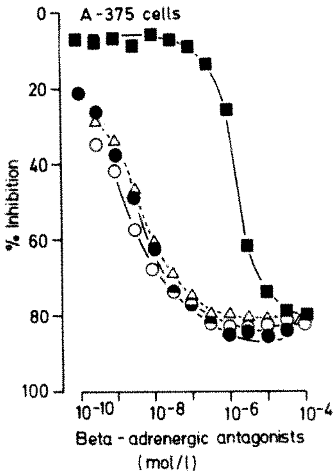

FIGURE 4. Inhibition of (−)-[^{3}H]CGP-12177 binding (1·0 nmol/l) to β-adrenoceptors on A-375 cells by the non-selective β-adrenoceptor antagonists propranolol and alprenolol, the β_2-selective antagonist ICI 118,551 and the β_1-selective antagonist bisoprolol. Each value is the mean of a single experiment performed in quadruplicate. The monophasic displacement pattern of all curves and the right-shift of the bisoprolol-curve by approximately three orders of magnitude indicate the existence of a homogenous β_2-subtype population of β-adrenoceptors on A-375 cells. ●, Propranolol; ○, alprenolol; △, ICI 118,551; ■, bisoprolol.

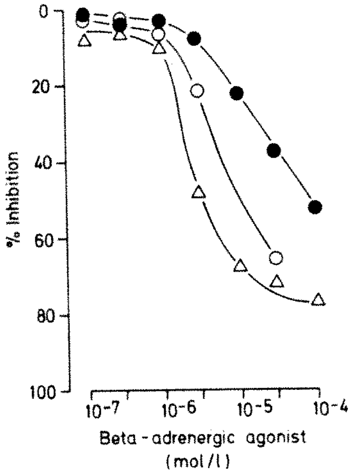

FIGURE 5. Inhibition of (−)-[^{3}H]CGP-12177 binding (1·0 nmol/l) to beta-adrenoceptors on A-375 cells by the β-adrenoceptor agonists isoprenaline, adrenaline and noradrenaline. Each value is the mean of a single experiment performed in quadruplicate. The existence of a β_2-subtype population in A-375 cells may be concluded from the agonist potency ranking: isoprenaline > adrenaline > noradrenaline. ●, Noradrenaline; ○, adrenaline; △, isoprenaline.

Antagonist displacement

Figure 4 demonstrates the competition by increasing concentrations of various antagonists with 1·0 nmol/l (−)-[^{3}H]CGP-12177 for the binding sites. The radioligand was displaced by the β-adrenergic antagonists in a concentration-dependent pattern. IC$_{50}$ values for the antagonists were (nmol/l): propranolol (non-selective) 2·8, alprenolol (non-selective) 2·0, ICI 118,551 (β_2-selective) 3·5 and bisoprolol (β_1-selective) 2200. Whereas the concentrations necessary to displace 50% of the radioligand from the binding sites are of the same order of magnitude for the two non-selective and the β_2-selective antagonists, the concentration of bisoprolol needed to displace the same amount of radioligand is 800 times higher.

Agonist displacement

β-Adrenergic agonists displaced 1·0 nmol/l (−)-[^{3}H]CGP-12177 from binding sites in a concentration-dependent pattern (Fig. 5). IC$_{50}$ values were (μmol/l): isoprenaline 3·2, adrenaline 6·3, noradrenaline 67·0. The potency ranking was assessed as isoprenaline > adrenaline > noradrenaline.

DISCUSSION

The characterization of β-adrenoceptors, their location on the cell membrane[21-23] and their activation after binding with agonists[3] have established that they are important models for the study of the nature and regulation of transmembrane signalling pathways. In this study we have demonstrated that intact cells of the human malignant melanoma cell line A-375 express β-adrenoceptors on their cell membranes. These receptors are at a similar density per cell as found in human polymorphonuclear leucocytes,[24] lymphocytes[25] and skin fibroblasts.[26] Binding studies on intact cells were considered to be more appropriate than studies on isolated membrane preparations.[4,25] However, intact cell binding is usually complicated by a large non-receptor related uptake into cells when lipophilic radioligands such as (−)-[^{3}H]dihydroalprenolol or (+/−)-[^{125}I] iodocyanopindolol are used.[24,27] It is probable that lipophilic radioligands interact with internal receptors and the use of the hydrophilic radioligand (−)-[^{3}H]CGP-12177 circumvents these problems and it is likely that only cell surface receptors are measured.[15]

The binding of (−)-[^{3}H]CGP-12177 to β-adrenoceptors on A-375 cells was of high affinity with a K_D of 0·25 nmol/l. This is in good agreement with the results of Staehelin *et al.*[15] who measured a K_D of 0·3 nmol/l for the binding of (−)-[^{3}H]CGP-12177 to β-adrenoceptors on intact C6 glioma cells. To determine how rapidly a radioligand binds to a receptor and dissociates from it, kinetic experiments must be performed and the association rate constant (k_{on}) and dissociation rate constant (k_{off}) must be derived from these studies. The quotient k_{off}/k_{on} will then yield the dissociation constant K_D. We have shown that the two K_Ds derived from these two different methods are in agreement (factor 2·7; $K_D = 0{\cdot}25$ nmol/l from saturation experiments; $K_D = 0{\cdot}67$ nmol/l from kinetic experiments).

Competition experiments revealed that the binding of (−)-[^{3}H]CGP-12177 to β-adrenoceptors was readily reversible in the presence of agents that compete for the same binding site. There is strong evidence for a β_2-subtype adrenoceptor on A-375 cells from our studies from antagonist displacement assays. While the non-selective antagonists propranolol and alprenolol as well as the β_2-selective antagonist ICI 118,551[20] were equally potent in displacing (−)-[^{3}H]CGP-12177 from the binding site, virtually none was displayed by bisoprolol at this concentration range. Bisoprolol is a very selective β_1-adrenoceptor antagonist.[18,19] It may displace ligands from β_2-adrenoceptors only when applied at very high concentrations[28] where drug selectivity is lost.[29] This was true in a concentration range that exceeded the concentrations of the other antagonists by three orders of magnitude. In many tissues[30,31], and also in a single cell type,[32] coexistence of β_1 and β_2-adrenoceptors has been described. Competition experiments with antagonists display a typical biphasic displacement curve.[29,33] In our experiments the monophasic displacement curves of all the antagonists indicated a homogeneous population of β_2-adrenoceptors on the A-375 cells.

The original classification of β-adrenoceptors into β_1 and β_2-subtypes was introduced by Lands *et al.*[34,35] and different orders of potency for sympathomimetic drugs on various tissues were determined. Additional evidence for the existence of a β_2-adrenoceptor subtype population on A-375 cells was provided by our experiments with agonist competition, which showed that β-adrenergic agonists displaced the radioligand with a rank order potency which corresponded well to their potencies on β_2-adrenergic systems.[34,35]

Binding studies with the human epidermal carcinoma cell line A-431 revealed that these cells had a homogeneous population of β_2-adrenoceptors.[36,37] The functional role of these receptors in A-375 melanoma cells is not yet determined. However, it is accepted that the binding of agonists to β-adrenoceptors stimulates the activity of the membrane-associated enzyme adenylate cyclase.[3,38] The functional coupling of β-adrenoceptors to the adenylate cyclase-cAMP-system in melanoma cells has been demonstrated[5] on B16 melanoma cells with isoprenaline. Due to the difficulty of culturing human melanocytes, the existence of β-adrenoceptors using radioligand binding studies has not been performed. As yet it is not known whether the expression of β-adrenoceptors is a general feature of all pigmented cells.

ACKNOWLEDGMENTS

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Integrins of normal human epidermis: differential expression, synthesis and molecular structure

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SUMMARY

The expression of integrin cell surface receptors in normal skin and their synthesis and molecular structure in keratinocyte cultures was investigated. The reactivity of four different polypeptides of the integrin family (α^2-, α^3-, α^6- and β_1-chains) was demonstrated in the basal cell layer of normal epidermis. Studies of labelled keratinocyte cell lines showed that the polypeptides were expressed as $\alpha^2\beta_1$, $\alpha^3\beta_1$ and $\alpha^6\beta_4$ integrins. Only the $\alpha^6\beta_4$ integrin showed polarization towards the basement membrane attachment site of basal layer keratinocytes, and was preferentially expressed at microvillous projections. In contrast, $\alpha^2\beta_1$ and $\alpha^3\beta_1$ integrins were equally expressed throughout the basal cell plasma membrane.

The integrins comprise a new super gene family of heterodimeric cell surface receptors involved in the cell–cell and cell–matrix adhesion of mammalian cells.[1,2] Several of these receptors were first identified and characterized by functional assays demonstrating their capacity to bind extracellular matrix proteins such as laminin, fibronectin and collagens or they were defined by monoclonal antibodies that block specific cell–cell or cell–matrix interactions. Four different groups of integrins have now been discovered which are characterized by the non-covalent heterodimeric association of one of four different homologous polypeptides (β_1–β_4-chains) with one of a larger group of homologous polypeptides (α-chains).[1-4] There is increasing experimental evidence that modulation of integrin receptors and their ligands is involved in a variety of physiological and pathophysiological processes such as differentiation, wound healing, inflammation and malignant transformation.[1,2] Recently it was reported that the extracellular matrix protein fibronectin had a marked effect on keratinocyte differentiation *in*

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vitro and that this was mediated by the integrin $\alpha^5\beta_1$, the cell surface receptor for fibronectin.[5] In normal adult epidermis this integrin was apparently not expressed.[6]

The constitutive expression of other members of the integrin gene family (VLA-2, $\alpha^2\beta_1$ and VLA-3, $\alpha^3\beta_1$, MAb J143) has been previously described.[6-9] Because integrins other than VLA-5 may also act as receptors that are important for the regulation of differentiation and cell–cell or cell–matrix interactions of keratinocytes we have analysed their reactivity in normal epidermis and studied their synthesis in keratinocyte cultures.

METHODS

Cells and culture conditions

Cell lines were cultured in RPMI 1640 supplemented with 10% fetal bovine serum (FBS), 2 mM glutamine, 1% non-essential amino-acids, 100 U/ml penicillin and 100 U/ml streptomycin. The cell lines Hacat[10] and the ras-transformed Hacat clone II/3[11] were gifts from Dr N.E.Fusenig (German Cancer Research Center (DKFZ) Heidelberg, F.R.G.). The melanoma cell line Brown was obtained from Dr M.R.M.van den Brink (Academisch Ziekenhuis Leiden, The Netherlands).

Monoclonal antibodies

Serum or ascites of hybridoma bearing nu/nu mice or tissue culture supernatant was the source of monoclonal antibodies (MAb): MAb TS2/7 binds to the α^1-chain of integrin $\alpha^1\beta_1$ (VLA-1),[7] MAb 10G11 and MAb Gi14 detect the α^2-chain of $\alpha^2\beta_1$ (VLA-2),[12] MAb J143 and VM-2 define the α^3-chain of $\alpha^3\beta_1$ (VLA-3),[13] MAb B-5G10 the α^4-chain of $\alpha^4\beta_1$ (VLA-4),[14] MAb B1E5 the α^5-chain of $\alpha^5\beta_1$ (VLA-5)[15] and MAbs GoH3 and MT78 recognize the α^6-chain of $\alpha^6\beta_1$ (VLA-6)[16] and of the $\alpha^6\beta_4$ epithelial integrin.[3] MAb Aj2 is directed to the β_1-chain which is common to all integrins of the VLA subgroup.[13] The hybridoma cell line secreting MAb MT78 was generated by immunizing BALB/C C57/BL6 F_1 mice with the ovarian cancer cell line SK-OV-4 using standard fusion and cloning procedures.[17]

Immunohistochemistry

Tissue specimens were snap frozen in liquid nitrogen, embedded in OCT-compound (Miles Laboratories, Naperville, IL, U.S.A.) and stored at − 135°C until tested. Cryostat-cut tissue sections (4–6 μm) were mounted on gelatin-coated slides, air-dried and fixed in cold acetone (4°C). Indirect immunofluorescence and immunoperoxidase tests were carried out as described previously.[9]

Radioimmunoprecipitation

Cells were metabolically labelled with [^{35}S]methionine (60–200 μCi/ml, New England Nuclear, Boston, MA, U.S.A.) for 12 h in methionine-free medium containing 10% dialysed FBS. Glycoproteins were isolated from Nonidet P40 solubilized cell extracts by adsorption to concanavalin A (Con A) (Pharmacia Inc., Uppsala, Sweden). Immunoprecipitations were carried out as described previously.[18]

RESULTS

We were primarily interested in the expression of extracellular matrix receptors by epidermal cells. Among the different subgroups of integrins, the β_1-subgroup, previously named the VLA-group, appeared to be particularly relevant to the dermo-epidermal junction (Table 1).

Expression of VLA-proteins in normal epidermis

The reactivity of the β_1-chain in normal skin common to all VLA proteins was analysed and a strong expression was found in the basal layer and weaker reactivity in the suprabasal layer. No expression was detected in the spinous and granular cell layers. Because the α-chains are thought primarily to determine the ligand specificity of the heterodimeric receptors (Table 1), monoclonal antibodies specifically directed to the α-chains were tested which were known to associate with the β_1-chain (α^1-α^6). Biopsies of normal skin from various regions of the body were studied ($n=45$). α^2-, α^3- and α^6-chains were expressed in the epidermal basal cell layer, whereas α^1-, α^4- and α^5-chain reactivity was not detectable. α^3- and α^6-reactivity was generally strong, whereas α^2 expression appeared more variable. α^6-reactivity was polarized towards the basal side of basal cells with much weaker reactivity at the lateral and apical sides of the plasma membrane (Fig. 1). In contrast, α^2- and α^3-chain reactivity was evenly distributed over the plasma membrane of basal cells (Fig. 1).

In the epidermal appendages, α^2-, α^3- and α^6-chain reactivity was again preferentially restricted to the basal cell layer and was not found, for example, in luminal cells of sebaceous glands or in the terminally differentiated keratinocytes of the outer hair root sheath. Also, the distribution of the different α-chains resembled that seen in the epidermis. The palmar and plantar epidermis of two young individuals was also studied. The epidermal basal cells had microvillous projections at their basal side that extended into the basement membrane. These microvillous projections are to be found in regions that are frequently exposed to mechanical stress and are considered to provide resistance to shearing forces.[19,20] They are barely visible on routine histology, but can readily be identified on electron microscopy. Anti-α^6-chain antibodies stained these structures intensely (Fig. 2). Anti- α^2- and α^3-chain antibodies also stained the basal cell microvilli but showed reactivity of similar strength with the other parts of the basal cell plasma membrane.

TABLE 1. β_1 and β_4-integrins: their known ligands and relevance for cell–cell and/or cell–matrix interactions

β_1-integrins	Ligands	Relevance for cell–cell and /or cell–matrix interaction
$\alpha^1\beta_1$ (VLA-1)*	Unknown	Unknown
$\alpha^2\beta_1$ (VLA-2)	Collagens	Cell–matrix
$\alpha^3\beta_1$ (VLA-3)	Laminin, collagens, fibronectin	Both
$\alpha^4\beta_1$ (VLA-4)	Fibronectin, VCAM-1	Both
$\alpha^5\beta_1$ (VLA-5)	Fibronectin	Cell–matrix
$\alpha^6\beta_1$ (VLA-6)	Laminin	Cell–matrix
β_4-Integrin		
$\alpha^6\beta_4$	Unknown	Unknown
? ⋮		

* Previously, the β_1-integrins were named VLA-proteins, since the first two heterodimers of this group were originally identified as very late appearing antigens on the T-cell surface after activation.

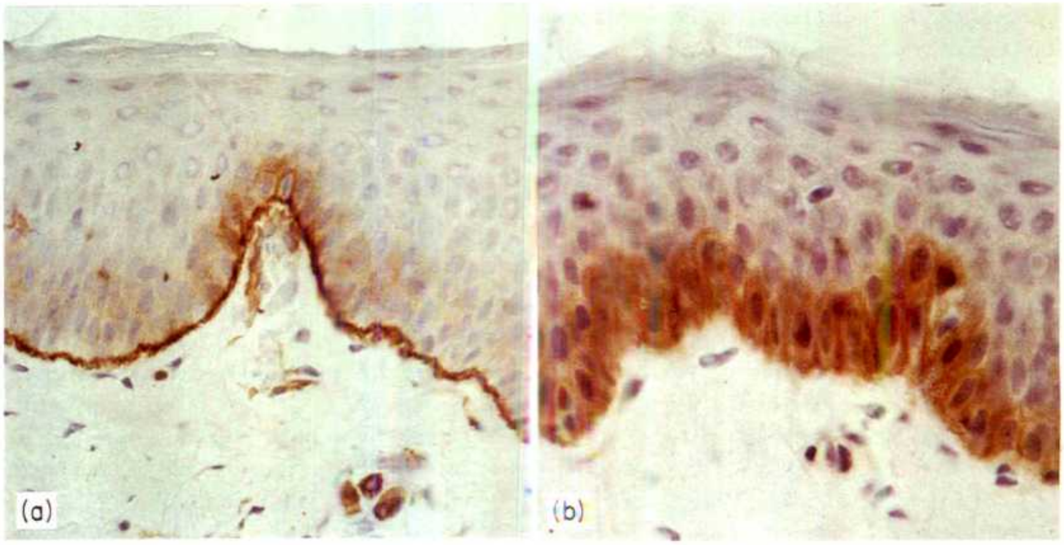


FIGURE 1. Expression of $\alpha^3\beta_1$ and $\alpha^6\beta_4$ integrins in normal epidermis. (a) Immunoperoxidase staining of frozen sections of adult human skin with monoclonal antibody GoH3 directed to the α^6-chain magnification, × 240. (b) Mouse monoclonal antibody J143 directed to the α^3-chain; magnification, × 378. Note the strong polarization of $\alpha^6\beta_4$ reactivity towards the basement membrane, which is not seen with $\alpha^3\beta_1$.

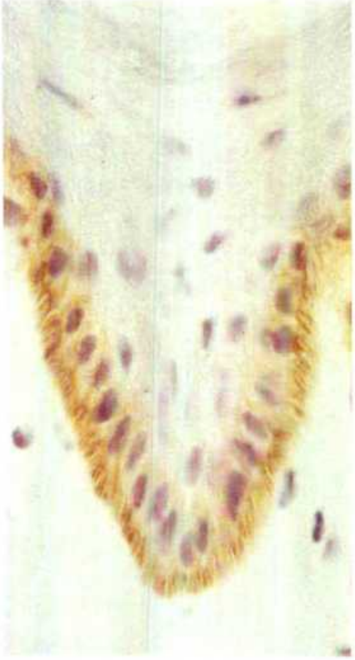


FIGURE 2. Preferential expression of the $\alpha^6\beta_4$ integrin at microvillous projections of basal cells. Immunoperoxidase staining of frozen sections of plantar skin of an 18-year-old individual with mouse monoclonal antibody MT78 directed to the $\alpha^6\beta_4$ integrin (magnification, × 410).

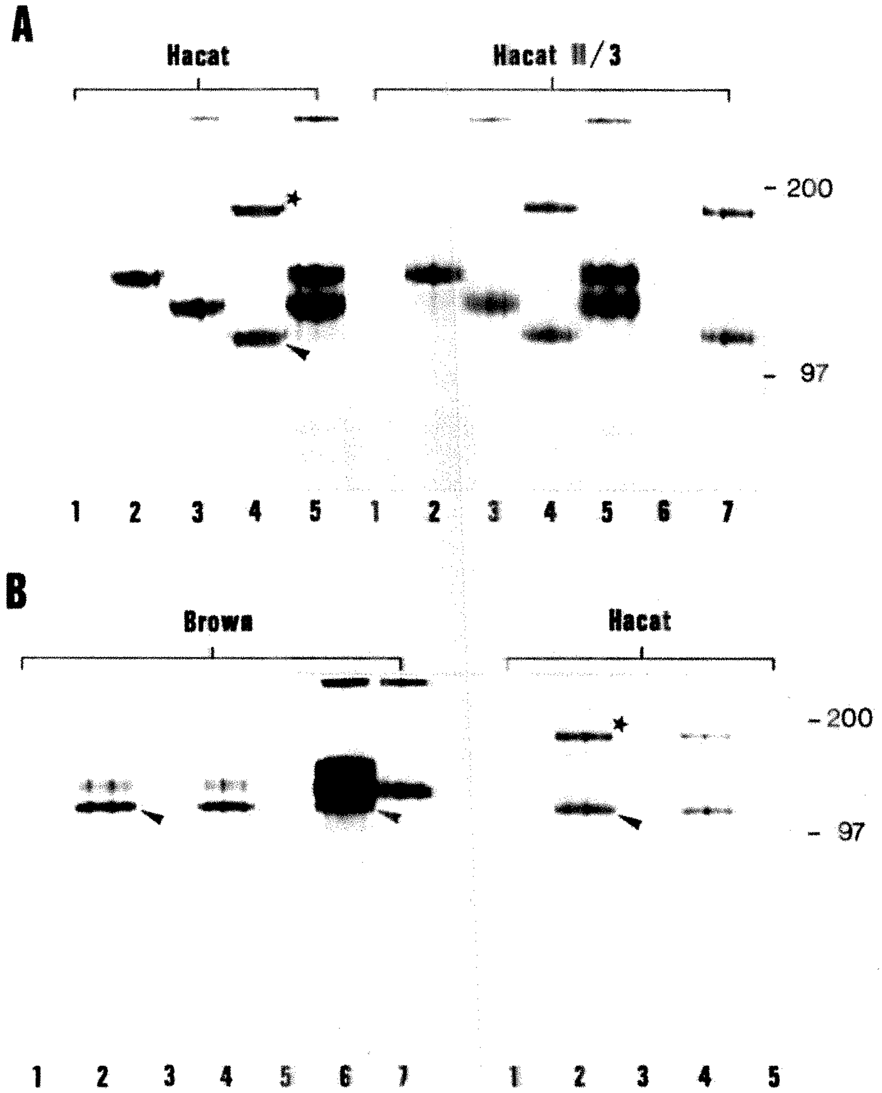
Synthesis of integrins in keratinocyte cell lines

The synthesis of integrins was studied using the immortal keratinocyte cell line Hacat and its oncogene-transformed derivative Hacat II/3. High synthetic levels of α^2-, α^3- and α^6-chains were found in both cell lines (Fig. 3a), whereas the synthesis of α^1-, α^4- and α^5-chains were not detectable (not shown). α^2- and α^3-chains were closely associated with the β^1-subunit (130 kDa), since both chains could be precipitated by the anti-β_1-subunit MAb Aj2 (Fig. 3a). Pretreatment with MAb Aj2 resulted in the removal of all α^2- and α^3-polypeptides from the Con A eluate (not shown). The α^6-chain, however, could not be precipitated by the anti-β_1 chain reagent MAb Aj2 (Fig. 3a) but was associated with another polypeptide of 200 kDa (Fig. 3a), since the anti-α^6-chain MAb GoH3 coprecipitated this 200 kDa protein together with the 120 kDa α^6-chain. A 200 kDa glycoprotein has recently been identifed as a novel β-chain of the integrin family and was designated β_4-chain. It was preferentially expressed in epithelial cancer cell lines[4] and was found in non-covalent association with the α^6-chain.[3] In conjunction with these results our immunoprecipitation data strongly suggest that in keratinocytes, the α^6-chain of integrins was associated with the β_4-chain to form this so-called 'epithelial' integrin. We have previously characterized several cell surface antigens of epidermal cells defined by MAbs raised against ovarian cancer cells (Klein *et al.*, unpublished). One antigen, defined by MAb MT78 has revealed the same tissue distribution in normal skin as the α^6-chain of integrins. Sequential immunoprecipitations from Hacat II/3 cells using MAbs GoH3 and MAb MT78 has shown that the novel MAb MT78 also recognized the $\alpha^6\beta_4$-integrin (Fig. 3b). Furthermore, sequential precipitations from the melanoma cell line Brown that expressed the α^6-chain in association with the β_1-chain showed that MAb MT78 was directed to an epitope on the α^6-polypeptide (Fig. 3b).

DISCUSSION

In this study we have demonstrated coexpression of three different integrins in normal epidermis. Two of them are composed of α-chains (α^2 and α^3) in association with β_1-chains and are selectively expressed in the basal cell layer, revealing reactivity which is evenly distributed over all aspects of the plasma membrane. The third integrin, the newly discovered 'epithelial' integrin which is composed of α^6-chains in association with β_4-chains, showed a marked polarization towards the basement membrane attachment sites of basal cells. This differential pattern of reactivity was confirmed by the use of two independently derived MAbs for each of the three α-chains. Moreover, the strong basal polarization of the $\alpha^6\beta_4$-integrin permitted the selective visualization of microvillous structures[19] at the basement membrane attachment site of epidermal basal cells. These microvilli are lost during the ageing process[20] and are considered to be important for anchoring the epidermis to the basement membrane and the underlying dermis. It is assumed that the $\alpha^6\beta_4$-integrin represents the laminin receptor of epithelial cells, since the α^6-chain in association with a β_1-chain has already been identified to function as receptor for laminin in platelets.[17] However, there is, as yet, no direct experimental evidence for this assumption.

Recently, in an ultrastructural study, we found that α^2-, α^3- and also β_1-chains (VLA-3 and VLA-2) were primarily associated with the intercellular contact sites of human tumour cell cultures, but apparently not with sites of cell–matrix adhesion.[13] In view of these results and the lack of polarization of α^2- and α^3-chains towards extracellular matrix proteins of the epidermal basement membrane, we suggest that β_1-chain-associated integrins of normal epidermis are primarily involved in the intercellular interactions of epidermal basal cells and to a lesser degree


A
Hacat
Hacat II/3
- 200
- 97
1 2 3 4 5 1 2 3 4 5 6 7
B
Brown
Hacat
-200
- 97
1 2 3 4 5 6 7 1 2 3 4 5

with adhesion and anchorage at the basement membrane. In contrast, the reactivity pattern of the $\alpha^6\beta_4$-integrin showing basal polarization and the preferential expression in basal cell microvilli supports the hypothesis that this integrin is primarily involved in substrate adhesion and anchoring of epidermal cells at the dermo-epidermal junction.

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FIGURE 3. Synthesis of integrins in keratinocytes and in a melanoma cell line. Fluorograms of immunoprecipitates obtained with MAbs were separated by SDS–PAGE (7·5%, section A and 9%, section B). Concanavalin A-bound fractions of nonidet P-40 extracts of [^{35}S]methionine-labelled cells were prepared as described.

(A) Synthesis of integrins was studied in the keratinocyte cell line Hacat and its ras-oncogene-transformed derivative Hacat II/3. Immunoprecipitates were obtained with MAbs as follows: normal mouse serum, control (lane 1); MAb 10G11 is directed against the α^2-chain and precipitates the $\alpha^2\beta_1$ integrin (lane 2); MAb J143 recognizes the α^3-chain and precipitates integrin $\alpha^3\beta_1$ (lane 3); MAb GoH3 is directed against the α^6-chain and precipitates the $\alpha^6\beta_4$ integrin (lane 4); MAb Aj2 recognizes the β_1-chain and precipitates all β_1 associated integrins—in this case $\alpha^2\beta_1$ and $\alpha^3\beta_1$ but not $\alpha^6\beta_4$ (lane 5); normal mouse serum (lane 6); MAb MT78 also precipitates the $\alpha^6\beta_4$ integrin (lane 7). Note that the α^6-chain (arrowhead) is not precipitated by the anti-β_1-chain MAb Aj2 but is associated with another protein of 200 kDa (⋆).

(B) Synthesis of α^6-chain-associated integrins was compared in the melanoma cell line Brown and the Hacat cell line. Immunoprecipitates were obtained as follows: normal mouse serum, control (lane 1); MAb GoH3 (lane 2); MAb MT78 after preclearing with GoH3 (lane 3); MAb MT78 (lane 4); MAb GoH3 after preclearing with MT78 (lane 5); MAb Aj2 (lane 6); MAb J143 (lane 7). Note that in the melanoma cell line Brown the 120 kDa α^6-chain (arrowhead) is associated with a 130 kDa polypeptide and is precipitated by the anti-β_1-chain antibody Aj2. In the keratinocyte cell line Hacat, the α^6-chain is associated with a 200 kDa polypeptide (⋆). In sequential immunoprecipitation, MAbs GoH3 and MT78 preclear each others' precipitates from the Con A-eluate indicating that they recognize the same molecule. Since this is the case for β_1-associated as well as for β_4-associated α^6-chains both antibodies must recognize epitopes on the α^6-chain.

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The extension technique: a new method of demonstrating initial lymph vessels in excised human skin

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SUMMARY

The initial lymph vessels, the lymph capillaries and precollectors, are difficult to detect in normal skin using routine histological methods. A method is described in which specimens of the skin are fixed in an expanded condition. In 1·05 μm thick plastic-embedded sections the dilated lymph vessels of the skin can be demonstrated. The dermal lymph vessel network can be seen following the subepidermal injection of a coloured solution. The endothelial cells and subendothelial fibrous network can be examined using electron microscopy.

There are only a few investigations into the density and distribution of the initial lymphatics (IL) of human skin. The difficulty is that these vessels possess very delicate walls that are completely collapsed in the skin biopsy specimens. Further understanding of the distribution of the IL will improve our understanding of the formation of lymphatic capillaries and the spread of tumour metastases in the lymphatics of different regions.

Drainage of the lymphatic fluid in the skin involves the lymph capillaries and precollectors. These vessels are to be found at the beginning of the lymphatic channel system and are referred to as the initial lymphatics (IL).[1,2] The lymph flows from the dermal network system into subcutaneous collectors. A dermal plexus is formed from the IL that consists of two horizontally running networks: a narrow-meshed superficial network lying subepidermally and a deeper wide-meshed network.[3,4] The networks are connected to each other through obliquely running precollectors.[3]

Using scanning electron microscopy the dilated dermal lymph capillaries were found to be 15–70 μm wide,[2,5] and consisted of an endothelial lined channel surrounded by a fibrous network. The elasticity of these vessels results from basal filaments and these IL are connected to the surrounding connective tissue by anchoring filaments. When the collection of interstitial

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fluid is increased, the connective tissue fibres are drawn apart and this exerts tractions on the IL wall and the lymph vessels dilate.[6–8] The IL has many interendothelial openings,[2,5,9] and the structure of their walls is different from that of the capillary blood vessels.[6] When dilated the precollectors are up to 150 μm wide and have connective tissue trabeculae and valves. The wall structure of these IL however largely corresponds to that of the lymph capillaries.[2,5]

We have developed a method in which the skin biopsies are mechanically dilated and fixed and the initial lymphatics visualized and assessed microscopically.

METHODS

Skin specimens

The skin biopsy specimens were taken from the margin of melanomas excised at a distance of 2–4 cm from the tumour. Skin biopsies were taken from 22 patients from various sites.

Extension method

The biopsy, consisting of a square-cut piece of skin approximately 1–2 cm^2 in size, was extended as shown in Figure 1. The specimen was then fixed in this state.

Macroscopic appearance of IL network

An aqueous Berlin blue solution (BBS) was injected subepidermally to reveal the IL network.

Light and electron microscopy

The skin biopsies were fixed using 4% glutaraldehyde with 0·2 M sodium cacodylate buffer (pH 7·3) for 24 h at 4°C. After post-fixation with osmium tetroxide they were embedded in Epon and

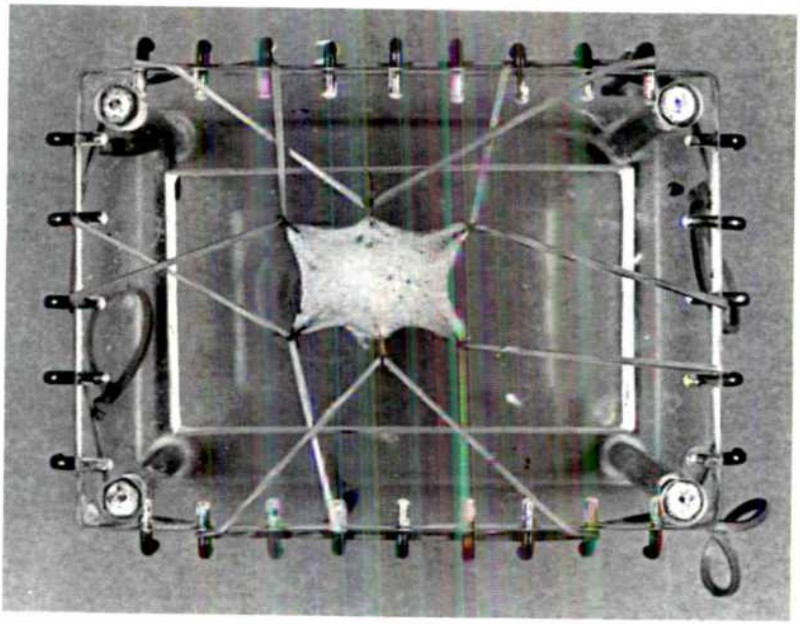

FIGURE 1. Method of extending the skin seen from above.

vertical sections 0·5 μm thick cut using a Reichart ultramicrotome. The sections were stained with 1% toluidine blue for light microscopy.

For transmission electron microscopy, ultra-thin sections were cut and stained with uranyl acetate and lead acetate. The sections were then viewed in the electron microscope.

Scanning electron microscopy

The specimens were fixed in 4% glutaraldehyde and after being rinsed in cacodylate buffer were then dehydrated and dried. The specimens were attached to an aluminium slide and using the sputter procedure (Polaron E 5000) vapourized with gold or gold–palladium. The scanning electron microscopy was carried out using a Philips SEM 505.

RESULTS

Extension

Preliminary studies showed that the skin specimens required different amounts of traction for the same degree of extension, varying according to their sites of origin. A good result was achieved when the pieces of skin were stretched so far that the surface appeared smooth and slightly shiny instead of being wrinkled. The subsequent histological and scanning electron microscopic studies indicated that the lymph vessels were partially opened by this extension. Rupture of the IL caused by the stretching was not seen.

Network appearance

Following the subepidermal injection of BBS visualization of the IL network was often achieved (Fig. 2). However, this was not always successful in all the specimens. The width of the meshed network varied considerably according to site, and in specimens from the head they were mostly less than 1 mm, whereas from other regions they were 0·5–3 mm. The diameter of the vessels

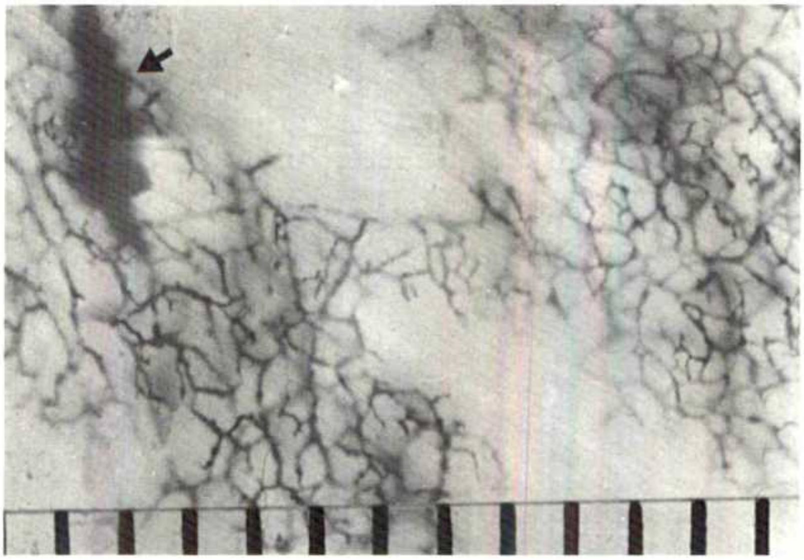

FIGURE 2. IL network following injection of Berlin blue solution. Skin taken from the left side of chest. Arrow: puncture point. Bar = 1 mm.

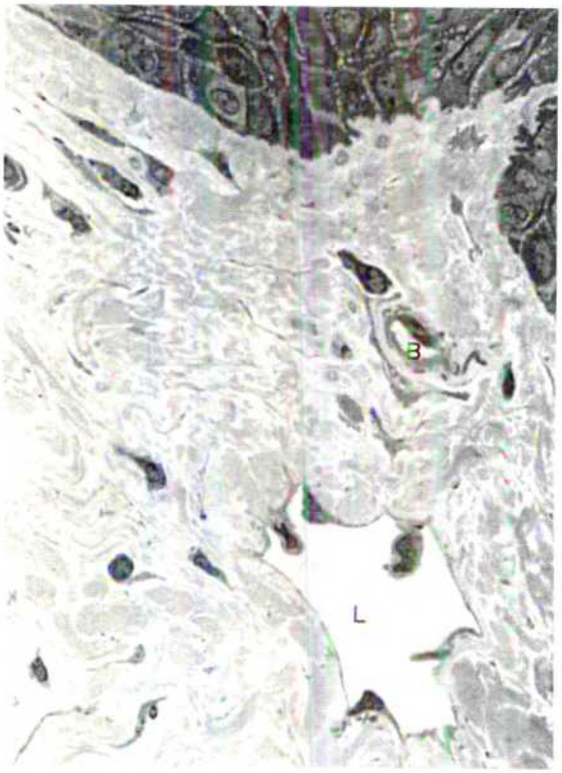

FIGURE 3. Initial lymphatic (L) with prominent endothelial nuclei protruding into the lumen; distance to the dermal/epidermal junction: approximately 40 µm. B = blood capillary. Skin of the back. Epon × 935.

varied from the superficial ones (50 µm) to the deeper parts of the network (100 µm). The networks were not usually apparent if the extension had been loosened before fixation. However, in preparations fixed in the extended condition the networks were clearly seen.

Histological results

In vertical sections the segments of the network filled with BBS could be seen. The vessels were only partially opened. Those IL in the lower papillary dermis parallel to the surface were consistently shown and isolated vessels just beneath the epidermic could be seen. In the deeper dermis only isolated lymph vessels could be seen, and these were to be found in the connective tissue adjacent to blood vessels and mainly running in an oblique vertical line. At the junction between the dermis and subcutaneous tissue, isolated lymph vessels were to be found mainly running in a horizontal direction. These vessels as a rule had a wider lumen than those in the upper dermis.

Extension preparations without dye

Using light microscopy, parts of the IL could be demonstrated in the vertical sections. These vessels were seen to have a single layer of endothelium and the nucleated portions of the cells protruded into the lumen (Fig. 3). In the papillary dermis the lymph vessels had a wider lumen than those of the blood vessels in that location. They could also be distinguished from post-capillary venules by a thinner wall, the lack of pericytes and the absence of erythrocytes.

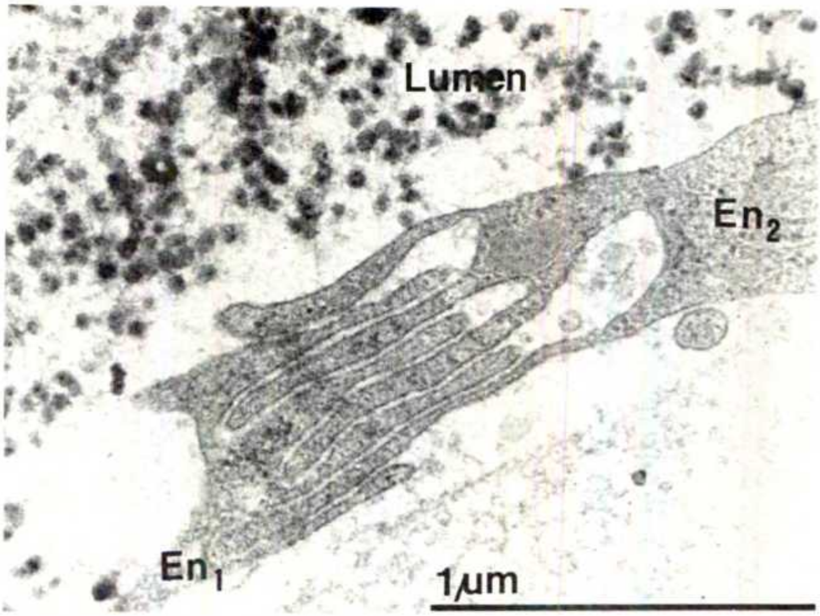

FIGURE 4. Transmission electron microscopy. Boundary between two neighbouring endothelial cells (En 1, En 2) of an initial lymphatic. The lumen is filled with Berlin blue solution (dark granules).

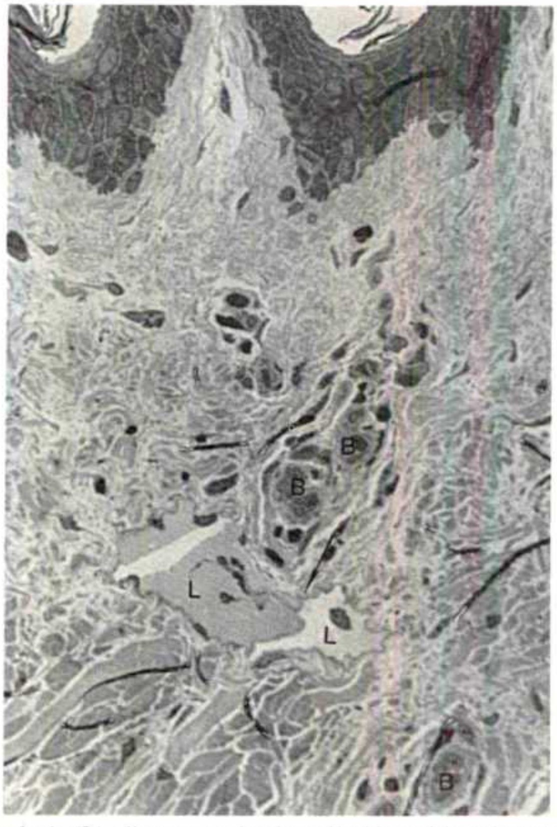

FIGURE 5. Initial lymphatic (L), distance to the dermal/epidermal junction: approximately 150 μm. B = blood capillary vessel. The arrow indicates a closed valve. The left part of the vessel cross-section is filled with lymph. Epon × 330.

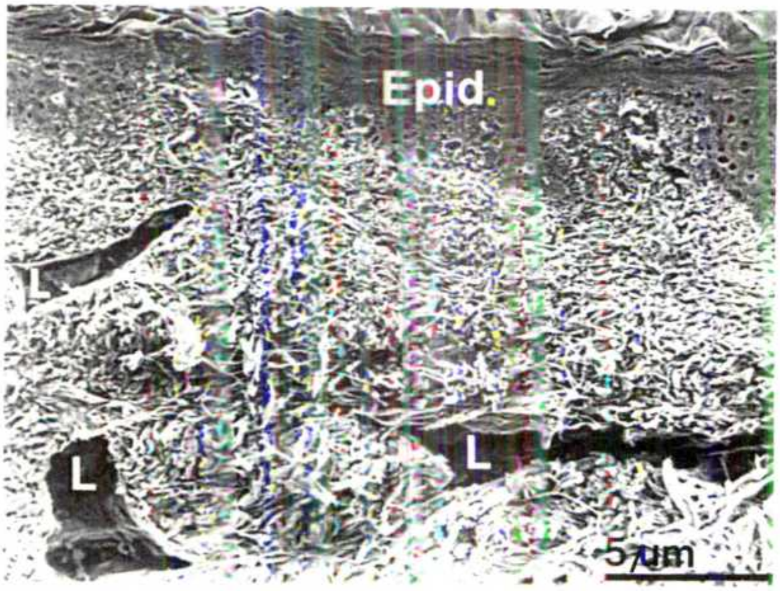

FIGURE 6. Scanning electron microscopy. Partially opened initial lymphatics (L).

Transmission electron microscopy showed an absence or partial presence of the basement membrane of the IL and a clearly visible subendothelial fibrous network (Fig. 4). Valves and trabeculae and interendothelial openings were demonstrated in the vessels in the dermis (Fig. 5). On scanning electron microscopy, the lumen of the IL could be readily identified (Fig. 6).

DISCUSSION

Our studies were prompted by the microlymph angiographic techniques as developed by Bollinger and co-workers who used a fluorescent dye in human skin.[10] In unstretched human skin biopsy specimens, injection of BBS failed to fill the lymph vessels which were collapsed. However, with the extension method which dilates the IL it was possible with the injection of BBS to demonstrate the vessels. For reasons that we cannot explain the injection of BBS to visualize the IL network was not always successful. Usually, parts of the network were filled via a single vessel and this took place within a few seconds. Our results have confirmed the horizontally arranged superficial network in the skin as has been shown by recent investigations by Kubik.[3]

The extension technique with the staining of the dermal IL has the advantage of making the lymph vessels in skin biopsy specimens visible without previous injection of dye. The method is suitable for both light and transmission electron microscopy, but less so for scanning electron microscopy. It is however unsuitable for routine histological investigation, as the skin biopsy specimens have to be rather large. We are currently modifying the technique for smaller biopsy specimens.

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Biochemical and trichological characterization of diffuse alopecia in women

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SUMMARY

One hundred women who presented with diffuse alopecia, were quantitatively evaluated for hair variables and compared with 20 controls. Fifty were selected for biochemical and haematological investigations, 44 of whom also underwent endocrine evaluation. Compared to controls, significant changes in hair values were found in the frontal area of all subjects, while 84% had significant changes in the occipital area. A biphasic distribution of hair diameter was evident in subjects who had percentages of vellus hair and telogen hair ≤ 30 mm in length that were above the control ranges. No significant difference between the mean hormonal values of women with diffuse alopecia and controls could be found. No correlation between hair values and individual or combined hormonal levels could be established. In 18 subjects (40·9%) hormonal values were within the control ranges and these apparently normal findings were often associated with adverse hair profiles. A raised dihydrotestosterone was found in 13 subjects (29·5%) and was the most frequently elevated androgenic finding. Seventeen (34·0%) had changes in iron metabolism, while in 36 (72·0%) serum ferritin levels were below the lowest control value. All had a decrease in the percentage of hair in the anagen growth phase compared to controls. The hair changes were similar to those observed in genetic hair loss in men, a proven androgen-dependent condition. We propose that diffuse androgen-dependent alopecia is the appropriate name to describe this condition in these women.

Thinning hair in men and women is predominantly a genetic condition which requires androgen mediation for its phenotypic expression (known as androgen-dependent alopecia, androgenic alopecia, androgenetic alopecia, common baldness, male pattern baldness, or female pattern baldness). The prevalence is almost 100% in men and around 30% in women, although precise epidemiological data are not available.[1] In women, the hair loss is usually diffuse and a pattern of the male type (marked loss of temporal or vertex hair) is less frequent.[2] Diffuse alopecia in

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women has been associated with endocrine abnormalities, but 30% or more have no demonstrable endocrine abnormality.[3–6] Only one study[7] presents quantitative data for scalp hair changes in relation to circulating hormonal values. Hypothyroidism reduces the proportion of hair follicles in anagen, hair diameter and hair density relative to a re-established euthyroid state.[8] Severe protein malnutrition is also known to cause major changes in hair growth.[9]

For most women, the major aesthetic change in diffuse alopecia is an apparent widening of the partings and a greater visibility of the scalp through the hair. Often the first realization that hair density is reduced occurs after an episode of increased hair shedding. Others experience only a gradual and insidious change in hair quality over many years. Most scalp hair disorders can be characterized quantitatively by analysing three features; hair density, diameter and length. The unit area trichogram[10] is a technique capable of providing such information.[11] The method has been employed to detail scalp hair changes during systemic anti-androgen therapy in women[7] and topical minoxidil treatment in men with male pattern baldness.[12] In view of the lack of quantitative trichological data in relation to biochemical and hormonal profiles, we used the unit area trichogram to investigate women in whom diffuse alopecia was their major, if not exclusive clinical feature.

METHODS

Selection of subjects with diffuse alopecia

One hundred pre-menopausal Caucasian females aged between 14 and 54 years (mean 32 years ± 8·5 SD) with diffuse alopecia, were investigated. Fifty, who attended consecutively, underwent biochemical and haematological investigations, 44 of whom also had endocrine evaluation. All subjects gave their informed consent. No patient had been taking iron, vitamin B_{12}, folic acid or multivitamin supplements for at least 3 months. No subject had suffered any illness lasting longer than 7 days, nor had they taken oral contraceptives, prescribed medications or applied products known to influence hair growth for 6 months. None had been pregnant within the previous 2 years and thyroid evaluations (TSH, free T_3 and free T_4) were all normal. None had been referred to an endocrine unit for obvious androgen excess or sought medical help for either acne or hirsutism. Subjects with alopecia areata, cicatricial alopecia or a history of thyroid dysfunction were excluded, as were those with an alopecia of the male type pattern.

Selection of control subjects

The 20 controls were Caucasian females aged between 17 and 49 years (mean 29 years ± 9·2 SD) with regular menstrual cycles, who were unaware of increased hair shedding or changes in hair quality or quantity during the previous 2 years. None had suffered illnesses lasting longer than 7 days, menstrual irregularities, gynaecological disturbances or had been pregnant. None had taken oral contraceptive preparations during the previous 6 months and none had ever sought medical advice for acne, hirsutism or scalp hair problems. From this group, 10 had biochemical, haematological and endrocrine evaluations.

The unit area trichogram

All subjects and controls followed the same standardized procedure over the 2 days prior to sampling, as detailed previously.[10] In addition, the interval between shampooing during the month prior to sampling was no longer than 2 days. The sample area was first cleansed with a 50% v/v acetone/isopropanol mixture to remove surface lipids. The sample area was marked through a rigid template using an Edding 77 rolling pen because circles drawn with fibre-tipped, felt-tipped or ball-point pens could not maintain the initial sharp line throughout the sampling period. The scribed area was measured from an enlarged black and white photograph. All hairs contained within and upon the scribed line were epilated singly with nasal forceps (#B885, J.Weiss Ltd., Milton Keynes, U.K.) in the direction of hair growth. Hair samples were obtained within an area 30–60 mm from the frontal periphery, 30–60 mm from the crown and 80–100 mm from the occipital periphery, 10–30 mm either side of the mid-line. The mid-line was defined as that extending from the nose to the occipital protuberance. Each hair was classified according to its growth phase and measured as detailed previously,[10] with the following modification.

The measurement of both the major and minor axes of every hair in the determination of hair diameter is too time consuming in the clinical situation. To alleviate this, hair diameters were not recorded if either axis was greater than 50 μm, as these hairs have mean diameters above the critical value of 40 μm.[10] Similarly, there was no need to measure both axes of hairs less than 32 μm as they are almost entirely circular in cross-section and rarely grow longer than 30 mm. These changes saved time without any apparent detrimental loss of information. However, in 23 subjects with diffuse alopecia and 11 controls, both axes were recorded and the averaged value employed to determine the frequency of hair diameters within these populations.

Hair variables employed in this study

The hair variables employed in this study are listed in Tables 1 and 2. Reproducibility was determined by undertaking two unit area trichograms, separated by less than 65 mm, mean 24 mm, from 30 females over a range of hair densities. No obvious clinical difference between the two sample sites of an individual was a criterion for sampling. Values from each site were pooled and the difference between this pooled value was employed to establish the mean percentage difference for each variable, which was less than 5% in each case. Consequently, mean values generated from the unit area trichogram are considered accurate to within ±5%. The term meaningful hair[10] was redefined by introducing a length limit. Only hair having a diameter >40 μm, or hair ≤ 40 μm in diameter longer than 30 mm was classified as a meaningful hair. This provided a working definition for terminal hair and consequently, hair ≤ 30 mm in length having a diameter ≤ 40 μm was classified as a vellus hair. The proportion of hair ≤ 80 mm in length provided an estimate of hair cycle disturbance during the previous 6 months. The percentage of telogen hair ≤ 30 mm in length was employed to assess complete growth cycles of less than 6 months. Where reference is made to hair length, this term relates to uncut hair only or cut hair above the pre-assigned limit. Subjects whose hair had been cut below these limits were excluded.

Dysplastic or dystrophic hair, i.e. hair that could not be readily recognized as anagen, catagen or telogen, was assigned to the anagen population if the root was bent, or otherwise classed as telogen. Catagen hairs were grouped with the telogen population for analysis. Less than 3% of hairs proved impossible to classify and the same observer (DHR) measured all the hairs.

Biochemical, haematological and hormonal evaluations

Biochemical, haematological and hormonal evaluations were undertaken in control and ovulatory subjects on day 21 of the menstrual cycle, before 11.00 and following a 12-h fast. In subjects with irregular menses investigations were delayed until menses occurred, whereupon a blood sample was taken 20 days later. Since these investigations spanned a 2-year period, control subjects were evaluated over this time and their blood samples were sent to the laboratory blind. Samples from controls and subjects were run together and the ratio of one control: five subjects was maintained as closely as possible. Unit area trichograms were undertaken on the same day as the hormonal, biochemical and haematological investigations, which are listed in Tables 3 and 4, respectively.

All hormonal, biochemical and haematological evaluations were undertaken by JS Pathology plc., London, U.K. For the hormonal and serum ferritin comparisons, values were compared to those obtained from 10 controls. The biochemical and haematological data could, however, only be compared with the reference range for the laboratory.

Statistical analysis

Group mean differences were compared statistically by non-parametric analysis employing the Mann–Whitney *U*-test or Student's *t*-test (independent samples) where appropriate. All analyses were undertaken on an Apple Macintosh™ computer using the statistical program STATWORKS™.

RESULTS

There was no significant difference between the mean ages of controls and subjects with diffuse alopecia ($P=0{\cdot}26$, Student's *t*-test-independent samples). The earliest reported awareness of reduced hair density occurred within 2 years of the onset of menstruation and by the third decade of life the majority of subjects had discernible diffuse alopecia. Irregular periods were noted in 16%, hirsutism in 12% and acne in 6%, but none of these conditions was troublesome enough to warrant any subject seeking medical advice. The mean number of hairs examined in controls was 114 from the frontal and 99 from the occipital, and from women with diffuse alopecia, 78 frontal and 81 occipital. A total of 17,082 hairs was examined in this study.

Distribution of hair variables in women with diffuse alopecia compared to controls

Values for hair variables, obtained from the frontal and occipital areas, were established in 20 controls and compared to values from women with diffuse alopecia (frontal $n=100$, occipital $n=62$, Tables 1 and 2, respectively). Significant differences in the grouped mean values for all hair variables occurred in the frontal and occipital areas. In 18 subjects vertex and frontal areas were also compared. No significant difference between these sites for mean values could be established (Wilcoxon signed rank test, data not presented). The severity of the alopecia was often, but not exclusively, related to age. Irrespective of age, the alopecia was always more severe in the frontal or vertex area, compared to the occipital. The percentage of subjects with values above or below the control ranges are also presented in Tables 1 and 2. Women with frontal hair densities within the control range had an increase in the percentage of hair $\leq 40\ \mu$m in diameter, ≤ 30 mm in length (vellus hair) and the percentage of telogen hair ≤ 30 mm in length, compared to controls.

Distribution of frontal hair diameter in 23 women with diffuse alopecia and 11 controls

Twenty-three women with diffuse alopecia were selected for examination of the distribution of hair diameter. In 11 subjects the percentages of vellus hair and telogen hair ≤ 30 mm in length were above the control ranges, while in 12 these values were within them. The distributions of hair diameter obtained from these two groups, as well as from 11 controls, are presented in Figure 1. A biphasic distribution was observed in the group of subjects in whom the percentages of vellus hair and telogen ≤ 30 mm in length were above the control ranges. In the group where these variables were within the control ranges the distribution of hair diameter was similar to that found in controls.

TABLE 1. Comparisons between controls ($n = 20$) and females with diffuse alopecia ($n = 100$) for frontal hair values and their frequency (%) above or below the control range

Variable	Controls	Diffuse alopecia	(%) Above	(%) Below
Total hair density cm^{-2}				
Mean ± SD	281 ± 41	***184 ± 49	None	82·0
Median	276	182		
(Range)	(234–376)	(69–320)		
Meaningful hair density cm^{-2}				
Mean ± SD	265 ± 41	***150 ± 41	None	90·0
Median	256	149		
(Range)	(212–345)	(58–266)		
(%) Anagen hair				
Mean ± SD	89·2 ± 3·0	***75·9 ± 10·4	None	82·0
Median	88·9	77·0		
(Range)	(85·7–95·7)	(44·9–93·6)		
(%) Meaningful anagen hair				
Mean ± SD	91·0 ± 2·6	***79·9 ± 10·6	None	71·0
Median	90·4	80·7		
(Range)	(86·5–96·2)	(48·6–95·9)		
(%) Hair ≤80 mm in length				
Mean ± SD	30·4 ± 7·2	***59·8 ± 14·3	86·0	None
Median	31·6	59·0		
(Range)	(19·6–42·7)	(25·9–92·0)		
(%) Vellus hair				
Mean ± SD	6·2 ± 3·0	***18·2 ± 8·5	67·0	1·0
Median	6·0	16·6		
(Range)	(1·1–13·0)	(0·0–39·7)		
(%) Telogen hair ≤30 mm in length				
Mean ± SD	2·9 ± 2·1	***13·7 ± 9·1	72·0	None
Median	2·3	11·8		
(Range)	(0·0–7·2)	(0·0–44·9)		

Significance *** $P < 0·001$ (Mann–Whitney *U*-test).

Biochemical, haematological and hormonal evaluations in women with diffuse alopecia compared to controls

From 100 women with diffuse alopecia, 50 underwent biochemical and haematological investigations, 44 of whom also had endocrine evaluation. The hormonal values were compared to those obtained from 10 controls and the percentage of subjects with values above or below the control ranges, are detailed in Table 3. Biochemical and haematological values above or below the reference range are given in Table 4.

Comparisons between hair values and hormonal values in 44 women with diffuse alopecia

Twenty-four subjects (54·5%) had one or more androgenic hormones (A-dione, DHEA-S, DHT, DHT/SHBG, Testo or Testo/SHBG) above or, in the case of SHBG, below the control range. Thirty per cent, 16·0% and 9·0% had two, three or four androgenic hormones invariant

TABLE 2. Comparisons between controls ($n = 20$) and females with diffuse alopecia ($n = 62$) for occipital hair values and their frequency (%) above or below the control range

Variable	Controls	Diffuse alopecia	(%) Above	(%) Below
Total hair density cm^{-2}				
Mean ± SD	279 ± 42	***229 ± 53	1·6	53·2
Median	266	226		
(Range)	(231–353)	(130–360)		
Meaningful hair density cm^{-2}				
Mean ± SD	256 ± 36	***201 ± 47	None	64·5
Median	247	200		
(Range)	(213–334)	(108–306)		
(%) Anagen hair				
Mean ± SD	89·4 ± 4·4	**83·3 ± 7·6	None	50·0
Median	89·1	84·4		
(Range)	(84·2–97·9)	(53·8–96·3)		
(%) Meaningful anagen hair				
Mean ± SD	91·4 ± 5·0	**85·7 ± 7·7	None	32·3
Median	91·9	86·2		
(Range)	(83·8–100·0)	(53·5–100·0)		
(%) Hair ≤ 80 mm in length				
Mean ± SD	34·9 ± 5·3	*42·7 ± 13·8	41·9	8·1
Median	33·9	41·8		
(Range)	(24·2–43·3)	(19·8–77·1)		
(%) Vellous hair				
Mean ± SD	8·1 ± 3·3	*12·5 ± 7·6	27·4	4·8
Median	8·5	10·3		
(Range)	(1·0–15·8)	(0·0–31·0)		
(%) Telogen hair ≤ 30 mm in length				
Mean ± SD	2·8 ± 2·1	**5·4 ± 3·8	30·6	None
Median	2·8	4·9		
(Range)	(0·0–7·5)	(0·0–15·0)		

Significance * $P < 0{\cdot}05$; ** $P < 0{\cdot}01$; *** $P < 0{\cdot}001$ (Mann–Whitney U-test).

to controls. Two (4·5%) had a raised prolactin level, one of whom required and subsequently responded to bromocriptine therapy. The remaining 18 subjects (40·9%) had no endocrine abnormality. No correlation between individual or grouped hormonal values and any hair value could be found. Of the 18 subjects with no endocrine abnormality, 16 (88·8%) had elevated levels of vellus hair and telogen hair ≤ 30 mm in length. Similarly, in 24 subjects with abnormal endocrine (androgenic) findings, 20 (83·3%) also had elevated levels of these hair variables.

In 13 subjects (23·1%) the percentage of meaningful hair in the anagen growth phase was within the control range. The distribution of these subjects with respect to endocrine abnormality was almost identical, six with and seven without abnormal endocrine findings.

TABLE 3. Day 21 hormonal values obtained from 44 women with diffuse alopecia compared to 10 controls

	SHBG (nmol/l)	DHT (nmol/l)	DHT/SHBG × 100	DHEA-S (μmol/l)	Testo (nmol/l)	Testo/SHBG × 100	A-dione (nmol/l)	Cortisol (nmol/l)	Prolactin (mIU/l)
Subjects									
Mean ± SD	52 ± 20	0·75 ± 0·44	1·61 ± 1·16	5·5 ± 3·4	1·42 ± 0·71	3·21 ± 2·45	4·7 ± 1·8	387 ± 155	170 ± 133
Median	53	0·64	1·38	4·9	1·32	2·60	4·7	355	145
(Range)	(20–112)	(0·20–2·10)	(0·27–6·88)	(0·9–18·0)	(0·30–3·30)	(0·54–11·0)	(1·9–11·0)	(145–927)	(25–846)
Controls									
Mean ± SD	58 ± 20	0·59 ± 0·19	1·43 ± 0·79	5·5 ± 3·2	1·33 ± 0·83	2·93 ± 1·61	5·0 ± 2·2	319 ± 88	113 ± 87
Median	65	0·62	1·17	5·7	1·15	3·17	4·8	297	92
(Range)	(29–81)	(0·30–0·88)	(0·65–3·03)	(0·7–11·0)	(0·30–2·90)	(0·81–4·30)	(1·9–8·4)	(212–509)	(25–314)
Significance	NS	NS	NS	NS	NS	NS	NS	NS	NS
Per cent of subjects with values above or below the control range									
(%) Above	6·8	29·5	11·4	6·8	4·5	22·7	6·8	13·6	4·5
(%) Below	13·6	2·3	6·8	None	None	4·5	None	6·8	None

NS, not significant ($P > 0·05$, Mann–Whitney *U*-test). Testo, Testosterone; A-dione, Androstenedione; DHEA-S, Dehydroepiandrosterone-sulphate; DHT, Dihydrotestosterone; SHBG, Sex hormone binding globulin.

TABLE 4. Percentage of biochemical and haematological values from 50 women with diffuse alopecia above or below the reference range

Variable	(Reference range)	(%) Above	(%) Below
Haemoglobin	(12·0–16·0 g/dl)	None	6·0
Folic acid (serum)	(5·0–40·0 nmol/l)	4·0	None
Folic acid (red cell)	(400–1600 nmol/l)	None	6·0
Vitamin B_{12}	(200–1000 ng/l)	None	None
Serum iron	(10–30 μmol/l)	4·0	12·0
TIBC	(45–70 μmol/l)	12·0	4·0
% Saturation	(20–55%)	None	24·0
Serum ferritin	(40–165 ng/ml)*	None	72·0
Transferrin	(2·0–4·0 g/l)	None	None
Serum zinc	(10·6–22·8 μmol/l)	None	None
Triglycerides	(0·6–2·0 mmol/l)	None	45·0

*Range given for serum ferritin was obtained from our 10 controls (see methods).

Comparisons between hair values and biochemical values in 50 women with diffuse alopecia

Adverse haematologial findings were observed in 12 subjects (24·0%), four of whom also presented with an iron imbalance. Thirty-six (72·0%) had serum ferritin values below the lowest control value (40 ng/ml) and in none was the percentage of hair in the anagen growth phase within the control range.

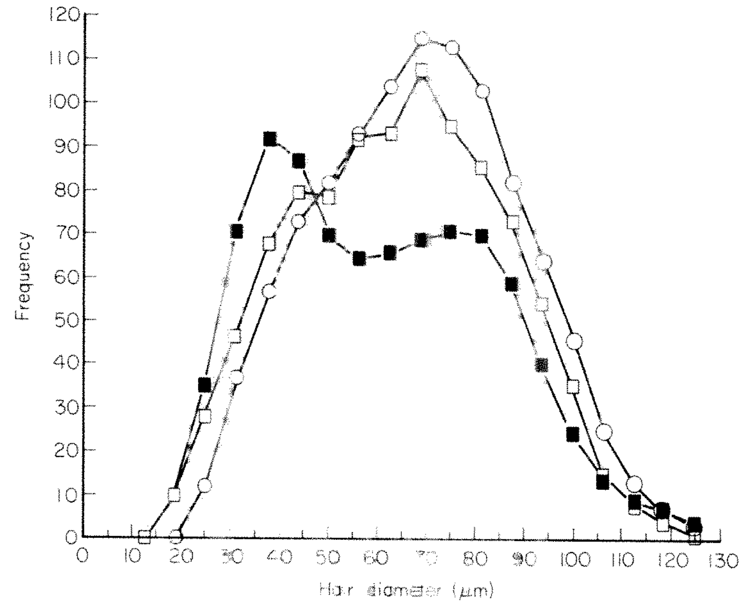

FIGURE 1. Frequency distribution of hair diameters (averaged major/minor axes) from 23 women with diffuse alopecia in whom the percentage of vellus hair and telogen hair ≤ 30 mm in length were above (active phase, $n = 11$) or within (stable phase, $n = 12$) the range established in 11 controls. ○, controls; ■, active phase; □, stable phase.

DISCUSSION

Hormonal analysis is frequently undertaken in women with diffuse alopecia. Miller *et al.*[3] found that 67% of subjects with diffuse hair loss had reduced SHBG levels compared to controls, suggesting increased free testosterone may be important in initiating diffuse hair loss. However, they excluded their lowest control value from their data analysis, the inclusion of which substantially reduced the percentage (20% vs. 67%) reported to have reduced SHBG levels. This lower frequency is much closer to the proportion we found (13·6%). In the present study we excluded subjects with male-type hair loss, whereas Miller and colleagues[3] did not, and this may account for the difference in results. In a group of 19 women with common baldness, Mortimer *et al.*[7] reported reduced SHBG levels in 68%. However, they employed the laboratory reference range, 50–80 nmol/l, whereas we established our own range (29–81 nmol/l) from a group of women without alopecia. We now believe that the lower limit of the reference range used in the Mortimer *et al.*[7] study was too high, and Miller and co-workers[3] quoted a similar range to ours (34–110 nmol/l) if their discarded value is included. Our findings for androstenedione, prolactin and testosterone were similar to those reported by Mortimer *et al.*,[7] as were the trichological values, suggesting diffuse alopecia and common baldness are the same condition. The findings of Kasick *et al.*[4] who studied 19 Caucasian women with diffuse alopecia, were also in good agreement with our data for SHBG, testosterone and DHEA-S with no significant difference found between subjects and controls for these hormones.

Recently two large studies have been published on the incidence of endocrine abnormality and diffuse alopecia. Futterweit *et al.*[5] investigated 109 women with mostly diffuse alopecia, while Moltz[6] studied 125 women with androgenetic alopecia exhibiting both diffuse and male type distributions. Comparisons between our data and those of Futterweit *et al.*[5] are difficult to make due to no ranges or medians being given. Moltz reported raised levels of DHT in 28% and for androstenedione in 6%, values almost identical to those we obtained of 29·5 and 6·8%, respectively. The frequency of change observed with other hormones was, however, two or three times higher. The number of subjects Moltz[6] reported with acne (41·6%), hirsutism (22·4%) and raised prolactin (34·0%) were significantly higher than ours, 6·0, 12·0 and 4·5%, respectively. Differences in patient selection may account for some of the discrepancy between our findings.

Biochemical and haematological differences have been found in women with diffuse alopecia compared to controls. In two studies conflicting data were presented with regard to iron metabolism,[3,6] and in one there was a substantial number of women (42%) with reduced serum ferritin.[6] In this study we observed an even higher prevalence (72%). The data in the study by Moltz was skewed and his reported median value of 41 ng/ml coincides with our lowest control value, which indicates that 50% of his group had reduced serum ferritin values by our definition. The remaining differences could be accounted for by weight and demographic influences. Clearly, little is known about the distribution of iron in peripheral tissue or its influence upon hair growth and this requires further investigation.

With quantitative hair data available, we have been able to assess the endocrine association with respect to fundamental hair variables. No correlation between hormonal levels and any hair value could be established. Hair changes expected to be associated with an adverse androgen response, increased frequencies of vellus hair and telogen hair ≤ 30 mm in length, were independent of circulating hormonal levels. This was particularly evident when comparing the trichological data with the levels of circulating DHT or the estimated free testosterone fraction (Testo/SHBG). Often DHT values as low as 0·4 nmol/l (control range 0·30–0·88 nmol/l) or a low Testo/SHBG ratio, were associated with increased percentages of vellus hair and telogen hair ≤ 30 mm in length. In some women with elevated circulating androgens, the frequencies of these hair measurements were within the control ranges. The degree of hair change occurring within the individual was independent of circulating hormonal levels. The endocrine dependence of diffuse alopecia appears to involve tissue dynamics such as receptor populations and binding protein phenomena, rather than specific levels of circulating hormones, supporting the view of Sawaya *et al.*[13]

From our data we propose that changes occur in diffuse alopecia as follows. In the initial stages there is a reduction in the proportion of hair follicles in anagen; 71·0% of subjects had reduced meaningful hair in the anagen growth phase. Correspondingly, the percentage of telogen hair ≤ 30 mm in length was elevated; 72·0% had raised values. As hair growth declines, an increase in the number of short hairs occurs; 86·0% had an increase in the proportion of hair ≤ 80 mm in length. Concurrent with these effects, although requiring a longer time scale, genetically orchestrated (androgen mediated) changes decrease hair diameter and increase the number of fine, short hairs; 67·0% of subjects had an increase in the proportion of vellus hair. Whether these changes occur during the anagen phase of the hair cycle or are activated between successive cycles, remains to be determined. Over time, these miniaturized hairs fail to emerge and as a consequence total hair density is reduced. A biphasic distribution of hair diameter was found in women in whom the percentages of vellus hair and telogen hair ≤ 30 mm in length were above the control ranges, but not in the group where these values were within them. Despite the problems of measuring hair diameter in a single plane, Jackson *et al.*[14] also observed a biphasic distribution of hair diameter in females without detectable endocrine abnormality. We propose that vellus hair frequencies of $> 13{\cdot}0\%$, and telogen hair ≤ 30 mm in length of $> 8{\cdot}0\%$, are diagnostic for diffuse alopecia in which reduced hair density will occur from the conversion of terminal hair to vellus hair. Diffuse alopecia also appears to have stable phases when the only features that may be evident are reduced total and meaningful hair densities.

Diffuse alopecia is not associated with any major endocrine disturbance, therefore extensive laboratory investigations would seem unnecessary in the majority of subjects. However, serum ferritin levels were reduced in 72·0% and this suggests that iron stores in non-essential tissue such as scalp hair may be more important than has so far been recognized. Recently, vitamin B_{12} levels have been shown to change adversely during anti-androgen (cyproterone acetate/ethinyl oestradiol) therapy.[15] Therefore, the following biochemical investigations could be undertaken as a basic screen for women with diffuse alopecia: free T_4 testosterone, serum ferritin, full blood count and vitamin B_{12} if anti-androgen therapy is to be considered.

In this study we have looked at the commonest type of hair loss affecting women in whom thyroid dysfunction had been excluded. The distribution of hair values had features similar to those observed in genetic hair loss in men, a proven androgen-dependent condition.[16] We propose that diffuse alopecia in females is also an androgen-dependent condition acting on a genetic predisposition. Therefore, diffuse androgen-dependent alopecia is a more appropriate name than androgenetic alopecia, androgenic alopecia, female genetic hair loss or common baldness, currently employed to describe diffuse alopecia. The necessity to demonstrate androgen excess before considering anti-androgen therapy would, in view of the trichological findings, appear inappropriate. Whether patients with or without androgen excess respond differently to anti-androgen therapy, remains to be established and is the subject of an on-going study.

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Susceptibility to irritants: role of barrier function, skin dryness and history of atopic dermatitis

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SUMMARY

The susceptibility of the skin to various irritants was investigated with the aim of determining the role of the barrier function of the stratum corneum, skin dryness and whether a history of atopic dermatitis (AD) was a factor. The transepidermal water loss (TEWL) was measured using an evaporimeter and skin hydration using a Corneometer and by visual scoring. The group with a history of AD ($n = 20$) had a lower pre-exposure barrier function and a higher TEWL value following irritant exposure than the group with a history of allergic contact dermatitis ($n = 18$) and a control group ($n = 18$). Clinically dry skin was more susceptible than normal skin, though no difference was noted in the pre-exposure barrier function. The increased susceptibility to irritants in those with a past history of AD was probably due to impaired barrier function and/or the presence of a dry skin.

Chronic irritant dermatitis includes those inflammatory skin reactions caused by repeated and cumulative damage to the stratum corneum by weak irritants.[1,2] Although the exact mechanism is unknown, it is likely that both endogenous and exogenous factors have important roles.[2,3] Characteristics such as a history of atopic dermatitis (AD),[3–6] dry skin,[6,7] and AD with dry skin,[3,8] a fair complexion,[9] impaired barrier function of the stratum corneum[10,11] and the existence of dermatitis on another part of the body[12] have all been reported to be associated with a higher susceptibility to irritants or risk factors for chronic irritant dermatitis. The purpose of this study was to determine the role of the barrier function of the stratum corneum, and whether skin dryness and a history of AD were important in the susceptibility of the skin to various irritants.

METHODS

Subjects

There were three groups of subjects.

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Atopic dermatitis group. Twenty patients with a past history of healed AD were selected. The diagnostic criteria for AD were according to Hanifin and Rajka,[8] and they had to be free of symptoms for at least 2 weeks prior to the experiment. The group consisted of four males and 16 females (age range 17–33 years, mean 25 years). The length of history of AD varied from 6 months to 21 years and the period when they were symptom free from 2 weeks to 6 years.

Allergic contact dermatitis group. There were 18 subjects with a past history of allergic contact dermatitis but with no previous history of AD and who had been free of symptoms for at least 2 weeks prior to the experiment. The group consisted of eight males and 10 females (age range 20–49 years, mean 31 years). The length of history of contact dermatitis varied from 4 months to 14 years and the period when they were symptom free from 1 month to 6 years.

Control group. There were 18 control subjects without any previous or present history of skin disease. The group consisted of 10 males and eight females (age range 20–35 years, mean 28 years).

Exposure

Aqueous solutions of 0·1% sodium lauryl sulphate (SLS), 2·3% di-sodium lauryl 3-ethoxysulphosuccinate (SUC) and 2·0% Shellsol K (SOL) were applied in 0·3 ml amounts to the skin on the proximal two-thirds of the volar aspect of the forearms. The concentrations were based on previous studies.[13]

Each solution was applied to a disc of absorbent Whatman paper which was fitted into a flat plastic chamber (Agfa Bayer, F.R.G.) with a wide brim and an inside diameter of 22 mm. On the right forearm the same chamber was used but without any solution. This served as a control. The distance between the exposed sites on each arm was 40 mm. Two applications lasting 45 min each were made on each working day during a period of 3 weeks (a total of 28 applications). The minimum interval between the two applications was 3 h. Following their removal the skin was cleaned with water and dried gently.

Evaluation

The measurements of TEWL were performed at each site on the first and fifth day of each week. The TEWL values at the exposure sites on day 1 before the first application were taken as the baseline value for these sites. For TEWL measurements the Evaporimeter EP1C (ServoMed AB, Vallingby/Stockholm, Sweden) was used.[14] The measurements were carried out after 15 min in an air-conditioned room at a constant temperature of 20°C and a relative humidity of 45 ± 5%, as has been described previously.[15]

The skin hydration state of the same sites was assessed using the Corneometer CM 820 PC[16] (Courage and Khazaka, Cologne, F.R.G.) immediately after the TEWL measurement on the first and fifth day of each week. On day 1, the forearm skin was scored visually for dryness, a positive score being given to those cases where the skin appeared and felt dry or rough. After exposure, the sites were graded visually according to Frosch and Kligman.[17]

Statistics

Analysis of variance was used for comparison of the following variables: mean TEWL and hydration values, mean age, mean length of dermatitis history, mean duration of symptom free period between the groups, mean measurement values on day 1 between the different sites and mean values of each site between the first and last day of the experiment. Differences were considered to be significant for a P-value $< 0{\cdot}05$. The χ^2 test was used to calculate the difference between the three groups with respect to sex and skin dryness. Multiple linear regression models were calculated for the TEWL values after exposure to the irritants as dependent variables and evaluated by the P-value of the partial F-tests.[18] In each regression model, various combinations of personal characteristics (such as baseline TEWL, skin dryness etc) were evaluated as independent variables.

RESULTS

The mean TEWL values for all subjects in the groups during the time course of the experiment and for the different sites are shown in Figure 1. The unexposed control site which was more distal to the exposed areas had a significantly higher mean baseline TEWL value (7·6 g/m²h) than the exposed areas (mean, 6·5 g/m²h). Each irritant caused a significant increase in the mean TEWL during the exposure period. No spatial variation in hydration values was observed on day 1. Throughout the entire exposure period no change in hydration could be discerned at any site. On day 19, a high total visual score ($\geqslant 2$) at the sites exposed to SLS, SUC and SOL was observed in 2, 2 and 0 individuals, respectively. In each case, these clinical signs were accompanied by a clear increase in TEWL ($\geqslant 5$ g/m²h).

The mean pre-exposure baseline TEWL value of the AD group was significantly higher than the other two groups (Fig. 2a), whereas the AD and contact dermatitis groups had lower baseline hydration values than the control group. Group AD had more subjects with a clinically dry skin than the other groups. There was a large variation in the individuals as regards the TEWL pre-exposure, but the intra-individual variation at the control site was low throughout the test period. The inter-individual differences in hydration values were also large, but there was a low intra-individual variation. No differences could be noted in the mean baseline TEWL values between subjects having a clinically dry or non-dry skin in the groups tested (Fig. 2b). However, the mean baseline hydration value in those with clinically dry skin was significantly lower than in individuals with a non-dry skin (means, 62 and 75 arbitrary units, respectively).

The AD group had a higher TEWL value than the contact dermatitis and control groups throughout the entire period at the exposed sites (Fig. 2a). Higher post-exposure TEWL values

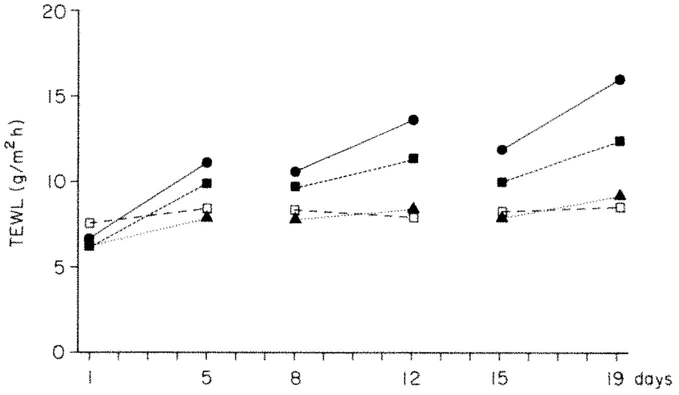

FIGURE 1. Mean transepidermal water loss values (g/m²h) at different sites over 3 weeks. Unexposed site □- - -□; irritant sites SLS ●——●; SUC ■- - -■; SOL ▲·····▲.

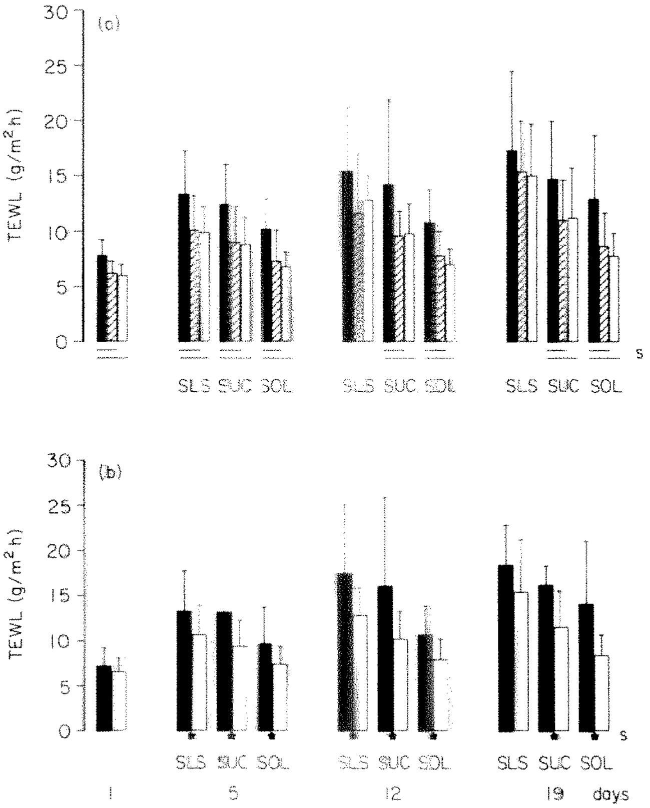

FIGURE 2. (a) Differences between groups AD (history of atopic dermatitis), CD (history of contact dermatitis) and C (controls) in mean transepidermal water loss value (g/m^{2}h) before and after exposure at different sites on various days. Group AD, ■; group CD, ▨; group C, □. s, denotes significance of difference ($P < = 0{\cdot}05$) between the groups. (b) Differences between subjects with a clinically dry and subjects with a non-dry skin in mean transepidermal water loss value (g/m^{2}h) before and after exposure at different sites on various days. Dry skin, ■; non-dry skin, □. s, denotes significance of difference ($P < = 0{\cdot}05$) between dry and non-dry skin.

TABLE 1. Correlation between baseline transepidermal water loss (TEWL) (g/m^{2}h) (BASE) at a site and TEWL value at the same site after various exposure time periods

Independent variable	Day	Site SLS	 SUC	 SOL
BASE	5	64*	52	55
BASE	12	27	28	53
BASE	19	10	18	36

* R^2 denotes the amount of variation in data explained by BASE (expressed as %).

TABLE 2. *P*-values of multiple regression analysis with transepidermal water loss (TEWL) on the fifth and the nineteenth day of exposure at different sites as dependent variables, and four combinations of baseline TEWL (BASE), dry skin (DRY) and history of atopic dermatitis (AD) as independent variables.

Combination of independent variables	Site		
	SLS	SUC	SOL
Day 5			
BASE	0·0001*	0·0001	0·0001
DRY	0·05	0·0006	0·05
BASE	0·0001	0·0001	0·0003
AD	0·9	0·4	0·7
AD	0·003	0·003	0·003
DRY	0·2	0·009	0·1
BASE	0·0001	0·0001	0·0002
DRY	0·05	0·001	0·06
AD	0·7	0·9	0·9
Day 19			
BASE	0·03	0·003	0·001
DRY	0·2	0·007	0·004
BASE	0·06	0·03	0·03
AD	0·8	0·2	0·3
AD	0·4	0·04	0·01
DRY	0·2	0·02	0·005
BASE	0·06	0·03	0·04
DRY	0·2	0·01	0·006
AD	0·8	0·5	0·5

* *P*-value denotes the significance of that variable's contribution to the model.

All dependent variables and BASE are continuous; AD and DRY are dichotomous.

were observed in the clinically dry skin than in the non-dry skin (Fig. 2b). Also in each group the dry skin had a higher susceptibility than the non-dry skin, and individuals with a non-dry skin having a history of AD were significantly more susceptible than those in the other groups.

The correlation between baseline TEWL and the values following exposure to irritants are shown in Table 1. For each irritant the correlation decreased as the time period of exposure increased. In several subjects the TEWL decreased after an initial increase up to day 5 or 12. Pre-exposure baseline TEWL made a highly significant contribution to the value of TEWL on day 5 (Table 2). This declined but remained significant up to the last day. A poor correlation existed between the baseline hydration determined by the Corneometer and the TEWL values following exposure.

DISCUSSION

For each irritant there was a moderate gradual increase in the mean TEWL over 3 weeks of exposure. In several subjects the TEWL curves had a descending slope during the last 1 or 2 weeks. This was probably due to an adaptation process. This may explain the declining correlation between baseline TEWL and TEWL after exposure to irritants over time. It is known that skin is able to adapt to chemical and physical irritation after a certain time.[1] The baseline TEWL as a direct reflection of the barrier function of the skin[14] may be more important during the first phase of the irritation process than later when adaptation occurs.

In AD, dryness of the skin is a common finding, and it is also seen in chronic irritant dermatitis,[6] in various skin diseases and in normal subjects without AD especially during the winter.[19] It can also be induced artificially by detergents and solvents.[13] It is unknown as to whether the functional aspects of barrier function and susceptibility to irritants are similar in these conditions. It has been reported that in AD patients the barrier function was related to the dryness,[20] whereas in those with ordinary winter xerosis the dryness was poorly correlated with barrier function.[19] In the present study in each group, clinically dry skin was more susceptible than non-dry skin, although no differences could be noted in the baseline measurements of the TEWL. Lammintausta *et al.* observed no difference in susceptibility between normal skin and skin that looked and felt rough.[21] However, in their study it is uncertain as to whether the site of exposure to SLS was the same as that scored for dryness.

The data indicates that a past history of AD increases the susceptibility to irritants. Previous reports have compared the susceptibility to irritants in those patients with different types of dermatitis that were manifest during the period of study.[5,22,23] However, that data from these studies is difficult to interpret since it has also been demonstrated that dermatitis itself may enhance susceptibility to irritants.[12] In the present study, subjects with a history of AD had a higher mean base TEWL than other subjects. This agrees with other studies.[5,20,24]

Multiple linear regression analysis permitted examination of the role of one factor such as dryness or AD in the presence of another factor, such as baseline TEWL. When baseline TEWL was in the model, a history of AD had a non-significant additional impact on the susceptibility values, whereas AD had a significant impact in the absence of baseline TEWL. Thus, the higher susceptibility of the group of AD patients can be explained, at least partly, by their higher baseline TEWL.

A dry skin in AD patients has been recognized as a risk factor for the development of hand eczema,[3] and in the present study, dryness of the skin was a significant factor in the susceptibility to irritants. Thus, the higher susceptibility in individuals with a past history of AD was probably also due to their skin dryness which was more frequently present in this group than the others.

Barrier function may not be the only factor which determines the susceptibility to irritants despite the high correlation. Reactivity of cellular targets underneath the stratum corneum[6] and the capacity for adaptation[1,2,25] may also be important. These latter parameters may account for the higher susceptibility in dry skin as has been demonstrated in the present study.

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Time-dependent inhibition of growth of human keratinocytes and fibroblasts by cyclosporin A: effect on keratinocytes at therapeutic blood levels

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SUMMARY

The effect of cyclosporin A (CyA) on the growth of cultured normal human keratinocytes and fibroblasts was studied. Tritiated thymidine incorporation per DNA content was used for short-term studies (1–2 days) and cell counting for longer term studies (up to 14 days). CyA caused a dose- and time-dependent inhibition of growth of both cell types. Keratinocytes (serum free, 70 μM Ca^{2+}) were more sensitive to CyA than fibroblasts (10% serum). For keratinocytes, at 2 days growth was 61% and 6% of controls with 3 and 10 μg/ml CyA, respectively, at 4 days growth was 51% with 1 μg/ml with complete inhibition at 10 μg/ml, and at 11 days growth was 58 and 39% with 0·1 and 1 μg/ml. For fibroblasts, at 4 days growth was 72 and 58% of controls with 10 and 30 μg/ml CyA, respectively, at 10 days growth was 76, 43 and 10% with 3, 10 and 30 μg/ml, and at 14 days growth was 41 and 11% with 3 and 10 μg/ml with complete inhibition with 30 μg/ml. For keratinocytes the effect of 24 h of CyA on growth was reversible. Growth of keratinocytes was inhibited by 10% delipidized serum, and 1% serum had a small stimulatory effect, but the action of CyA was unaltered. We conclude that increasing the duration of exposure to CyA increases the concentration-dependent growth inhibition. At therapeutic blood levels of CyA the growth of cultured keratinocytes but not fibroblasts is inhibited. The benefit of CyA in reducing the hyperproliferation of psoriasis may include a direct effect on keratinocytes.

Cyclosporin A (CyA), a cyclic polypeptide of fungal origin widely used as an immunosuppressive agent, has recently been shown to be effective in the treatment of several dermatological diseases.[1,2] Many of these diseases have an immune basis, notably atopic eczema,[3] pemphigus vulgaris,[4] bullous pemphigoid[5] and Behçet's disease.[6] The effectiveness of CyA in the

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prophylaxis of organ rejection is thought to be due to the inhibition of proliferation of helper T cells. The inhibition of the production and release of cytokines that is attributed to CyA may be important in the therapeutic effect of CyA in immune-mediated diseases.[7] However, CyA is also of proven efficacy in psoriasis where the mechanism of epidermal hyperproliferation is not yet established.[8,9] Although inhibition of cytokine release may be important, there is evidence suggesting CyA may have a direct effect on keratinocyte growth.

A recent study using human skin transplanted onto athymic nude mice demonstrated reduced DNA synthesis of keratinocytes after 3 weeks of subcutaneous injection of CyA.[10] Serum levels of CyA were within the therapeutic range. Most studies of the *in vitro* effect of CyA on the growth of human keratinocytes have used short-term cultures of 4 days or less. We have investigated the effect of CyA on the growth of normal human keratinoytes and fibroblasts *in vitro* for culture periods of up to 14 days. We have also investigated the effect of serum on keratinocytes as it has been reported that it reduces the sensitivity to growth inhibition by CyA.[11]

METHODS

Cell culture

Human keratinocytes and fibroblasts were grown from normal skin obtained from circumcisions or retro-aural skin removed during plastic surgery. The skin was trimmed of fat, cut into strips and incubated overnight at 4°C in phosphate-buffered saline (PBS) containing dispase (Boehringer Mannheim, Sussex, U.K.) 2 mg/ml. The epidermis was separated with fine forceps, incubated for 10 min at 37°C with trypsin (0·05%) and EDTA (0·02%) and gently shaken to disaggregate the basal keratinocytes. Trypsin was removed by washing and the cells plated on to plastic wells and flasks (Costar) in a serum-free medium MCDB153 (Sigma Chemical Co., Poole, Dorset, U.K.) that included calcium at a concentration of 70 μM and insulin (5 μg/ml), hydrocortisone (5×10^{-7} M), transferrin (5 μg/ml), and epidermal growth factor (10 ng/ml). When the cultures were near confluence cells were removed by trypsinization and seeded with the addition of bovine hypothalamic extract to enhance attachment. Eight keratinocyte strains were studied, using passage 1–5 in addition to primary cultures.

Three human fibroblast lines were established by explant culture from a dermal portion of a skin specimen. Cells were grown in DMEM (Northumbria Biological Ltd., Cramlington, U.K.) with 10% fetal calf serum (FCS) and used from passage 4–12.

Cell growth

To study the short-term effect of CyA on cell growth (1 or 2 days), [^{3}H]-thymidine incorporation was measured since this is more sensitive to early changes than cell number. Cells grown in 35-mm wells were labelled for 2 h by addition of [^{3}H]-thymidine 5×10^{-7} M, 2 Ci/mmol (Amersham International, Aylesbury, Bucks, U.K.). A 20-fold excess of unlabelled thymidine and deoxycytidine was added to flood the nucleotide pool. After incubation the cells were washed with PBS (×2), 5% trichloracetic acid (×5), ethanol (×2) and dissolved in alkali (0·3 M NaOH). Aliquots were taken for scintillation counting or neutralized prior to DNA measurement using the fluorescent dye bisbenzimidine (H33258). Samples were added to 75 mM citrate buffer (pH 7·0) with a final dye concentration of 1 μg/ml using calf thymus DNA as a standard. Results were calculated as dpm per μg DNA and expressed as percentage of control cultures.

For longer term studies cell counting was used. Cells were seeded on to 35-mm wells and after

24 h duplicate wells were trypsinized and counted to determine the number of cells attached. The cells were grown for up to 11 days in the presence of CyA and after trypsinization and dispersion into a single cell suspension counted with a Coulter counter model ZM. Keratinocytes grown in low calcium conditions do not form desmosomes and a cell suspension was easily obtained. In control studies a good correlation was seen between cell counts by counting chamber and the Coulter counter.

Materials

Cyclosporin A (a gift from the Sandoz Corporation) was dissolved in ethanol at 10 mg/ml and stock solutions prepared in ethanol. FCS (Biological Industries, Cumbernauld, Scotland, U.K.) for use in some of the keratinocyte cultures was delipidized to avoid partitioning of CyA within the lipid fraction of the serum. The serum was treated with an equal volume of chelex TM100 (Biorad) for 20 min with gentle agitation to remove the calcium. After filtration the supernatant was added drop-wise to 10 volumes of acetone/ethanol (1 : 1) at 4°C. The precipitate was washed with an equal volume of cold ether and when dry resuspended in the original volume of double distilled water and filter sterilized. Other chemicals were obtained from Sigma.

Statistical analysis

Student's unpaired *t*-test or analysis of variance was used to determine the statistical significance of differences between group means.

RESULTS

Cyclosporin A caused a dose-dependent inhibition of [^{3}H]-thymidine incorporation by keratinocytes in short-term growth studies at 1 and 2 days (Fig. 1, pooled results from all eight lines). Thus, after 2 days, incorporation was reduced to 61% of control values ($P < 0{\cdot}02$) by 3 μg/

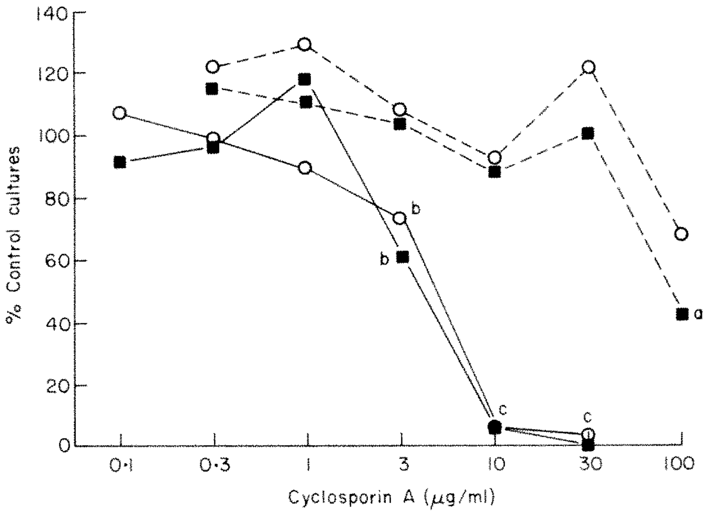

FIGURE 1. Effect of cyclosporin A for 1 (○) and 2 (■) days on thymidine incorporation of human keratinocytes (——) and fibroblasts (– – – –). Results are corrected for DNA content and expressed as % of control cultures (no CyA). Significant differences from control cultures ($n = 3$–7): a = $P < 0{\cdot}02$, b = $P < 0{\cdot}01$, c = $P < 10^{-4}$.

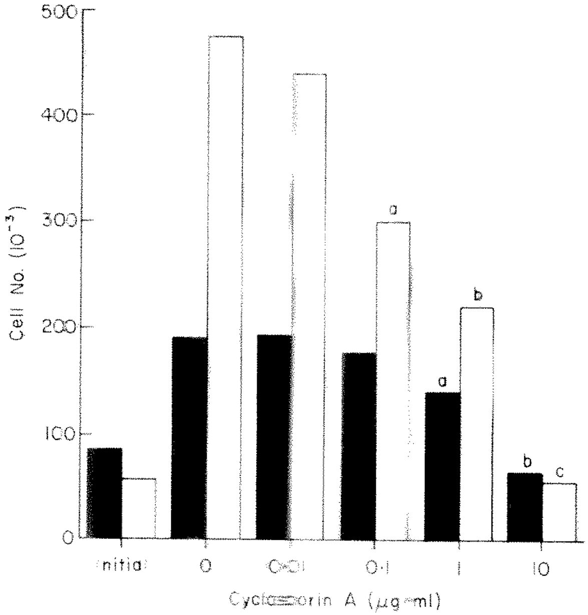

FIGURE 2. Concentration effect of cyclosporin A on keratinocyte growth at 4 and 11 days. The cell number at the start of CyA treatment is shown as Initial. Significant differences from no CyA cell number ($n = 3$–5): a = $P < 0{\cdot}05$, b = $P < 0{\cdot}01$, c = $P < 0{\cdot}001$. ■, 4 days; □, 11 days.

ml CyA and to 6% by 10 μg/ml ($P < 10^{-4}$). By contrast, fibroblasts at 2 days were resistant to growth inhibition at less than 100 μg/ml CyA and then only showed inhibition to 43% of control ($P < 0{\cdot}02$). In both cell types although there was a trend to greater inhibition at 2 days, this was not statistically significant.

Longer term keratinocyte cultures, grown in the presence of CyA, showed both a dose-and time-dependent inhibition of growth. Thus, after 4 days there was significant inhibition of growth (increase in cell number from initial seeding) to 51% of the control at 1 μg/ml and complete inhibition at 10 μg/ml CyA (Fig. 2). By 11 days, inhibition of growth of keratinocytes was to 58% ($P < 0{\cdot}05$) and 39% ($P < 0{\cdot}01$) of control cultures with 0·1 and 1 μg/ml CyA. Fibroblast growth was only inhibited by higher concentrations of CyA. Thus, after 4 days fibroblasts were significantly ($P < 0{\cdot}02$) inhibited at 10 and 30 μg/ml CyA (Fig. 3). At 10 days there was inhibition to 76% ($P < 0{\cdot}01$), 43% ($P < 10^{-6}$), and 10% ($P < 10^{-8}$) of control cultures with 3, 10 and 30 μg/ml CyA, respectively. In a 14-day experiment there was inhibition to 41% ($P < 0{\cdot}001$) by 3 μg/ml CyA with greater effects at higher concentrations. Similar results were obtained with other lines established (data not shown).

Delipidized serum added to keratinocyte cultures had different effects depending on the concentration. Thus, 1% produced a small but significant ($P < 0{\cdot}01$) stimulation of growth after 1 day (Fig. 4), whilst the concentration of serum used in fibroblast cultures (10%) was found to inhibit the growth of keratinocytes. In the presence of 1% serum the CyA-mediated inhibition of [^{3}H]-thymidine incorporation was unaffected. To see if the effect of CyA was reversible it was washed out of cultures of keratinocytes after 1 day. [^{3}H]-thymidine incorporation determined 24 h after washout was not reduced (Fig. 4), showing that the short-term effect of CyA on growth was reversible.

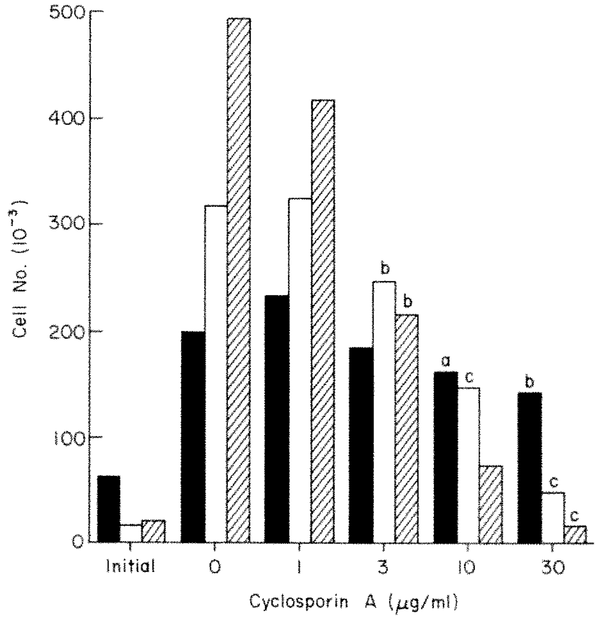

FIGURE 3. Concentration effect of cyclosporin A on fibroblast growth at 4, 10 and 14 days. The cell number at the start of CyA treatment is shown as Initial. Significant differences from no CyA cell number ($n = 3$–5): a = $P < 0{\cdot}02$, b = $P < 0{\cdot}01$, c = $P < 10^{-5}$. ■, 4 days; □, 10 days; ▨, 14 days.

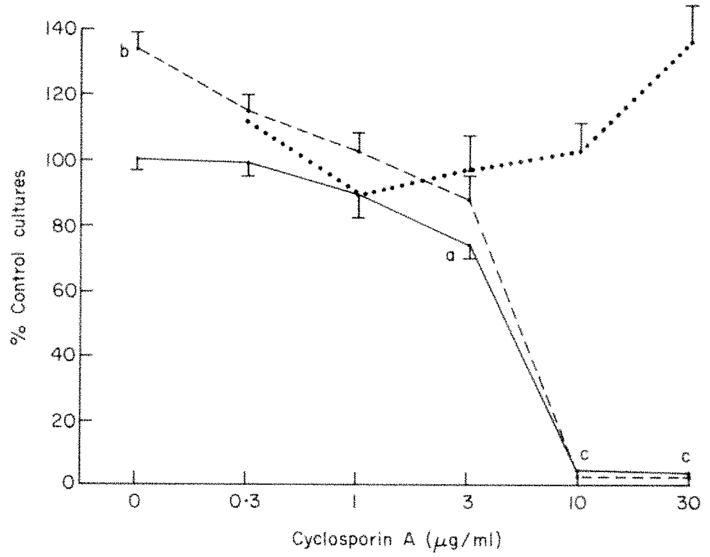

FIGURE 4. Effect of 1 day of cyclosporin A on thymidine incorporation of keratinocytes (——), 1% serum (- - - -), and 1 day after CyA washout (• • • • •). Results are corrected for DNA content and expressed as % of control cultures (no CyA or serum). Significant differences ($n = 4$–6): a = $P < 0{\cdot}02$, b = $P < 0{\cdot}01$, c = $P < 10^{-4}$.

DISCUSSION

The results have demonstrated a dose- and time-dependent inhibition of the growth of cultured human keratinocytes and fibroblasts by CyA. Keratinocytes are more sensitive to this effect than fibroblasts and in longer term experiments (11 days) are inhibited at concentrations of CyA which fall well within the usual therapeutic range. Other workers have examined this effect and although there are some inconsistencies, the published data suggest a time-dependent response.

An early study of epidermal cell outgrowth from explanted pig skin suggested that CyA for 3 days did not inhibit epidermal growth at therapeutic serum levels.[12] A more recent study[10] of the *in vivo* antiproliferative effect of 3 weeks of CyA on human keratinocytes grafted onto nude mice, found inhibition with 679 ng/ml CyA (2 h after administration, $n=3$). This is similar to the therapeutic range used in the treatment of psoriasis where the trough blood levels (12 h after the last oral dose) are 200–600 ng/ml.[8,9] In a recent study on epidermal proliferation in the unaffected skin of psoriatic subjects, CyA had no effect on the increased mitotic rate following cellophane tape stripping.[13] The abnormally rapid nail growth of psoriatics was significantly inhibited by CyA, but unaltered in non-psoriatics.

Normal mouse keratinocytes investigated *in vitro* demonstrated inhibition by 10 μg/ml CyA of DNA synthesis measured by thymidine incorporation at 24 h.[14] Investigation of transformed keratinocyte cell lines suggested that the effect of CyA may depend on both the concentration and the duration of exposure to the drug. Significant inhibition was reported of the growth of human keratinocytes in serum free medium containing CyA 5 μg/ml for 4 days.[11] With longer term cultures, the inhibitory effect was seen at lower concentrations (1 μg/ml CyA for 8 days).[15] These results were confirmed by the present findings and were extended to show that at 11 days 0·1 μg/ml CyA inhibits keratinocyte growth. We also confirm the report[15] of the reversibility of the action of CyA on short-term cultures of keratinocytes. There are few studies of epidermal concentrations of CyA, but levels have been reported to be higher than those found in blood.[11] The significance of the higher level of CyA in the epidermis is not clear, but certainly sufficient CyA is present to have a direct effect on keratinocyte growth.

As with keratinocytes, fibroblasts showed increasing inhibition of growth with increasing duration of exposure. At 4 days there was inhibition by 30 μg/ml CyA and at 10 days, 3 μg/ml CyA was inhibitory. These results confirm the conclusions of Nickoloff *et al.*[16] that fibroblasts grown in serum-containing medium are much more resistant to the action of CyA than keratinocytes grown in serum-free conditions. This may be due to different susceptibilities in different cell types. However, it has also been suggested that whole serum has a protective effect,[11] but delipidized serum does not.[16]

In the serum-free medium MCDB153 the addition of 10% chelex-treated delipidized serum itself inhibited keratinocyte growth. The addition of 1% serum had a small but significant stimulatory effect on DNA synthesis in keratinocytes, but did not protect against the effect of CyA at 24 h. This is in agreement with the lack of protection from 10% delipidized serum with fibroblasts.[16] The partitioning of CyA within the lipid fraction of serum may explain this action.

The hyperproliferative state of the psoriatic epidermis has similarities to the proliferation of keratinocytes in culture with expression of the hyperproliferative 48 kDa and 56 kDa keratins (K6, K16).[17,18] An investigation of the effect of CyA on the growth of human cells separated from systemic influences, including the immune system, is important in understanding the action of CyA on the skin and in the treatment of psoriasis. CyA, within the therapeutic dose range, has been shown to have a direct inhibitory action on the growth of keratinocytes in culture. Although the action of CyA in psoriasis may be mediated by regulatory cytokines and other aspects of the immune system,[19] a direct effect on the epidermis may also be important. In a number of cell types, including lymphocytes, CyA has been shown to inhibit Ca^{2+}/calmodulin-induced effects,[20] and regulation of intracellular pH;[21,22] these early effects may be common to many cell types and be important in inhibiting proliferation.

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Squamous cell carcinoma of the nail bed: a clinicopathological study of 12 cases

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SUMMARY

Twelve cases were reviewed of squamous cell carcinoma of the nail bed, with emphasis on the aetiological role of human papillomavirus (HPV) infection and radiation damage. Using a hybridization technique, similar HPV genomes were detected in a uterine cervical neoplasia and a subungual squamous cell carcinoma in the same patient.

Although squamous cell carcinoma (SCC) is the most common tumour found in the nail bed,[1] it is an uncommon lesion. Only 12 cases have been seen in 27 years at Memorial Sloan-Kettering Hospital[2] and 12 in our institution during the last 10 years; both being large referral centres with about 30,000 surgical specimens a year. Since it was first described in 1850,[3] almost 100 cases have been reported.

The cause of SCC of the nail bed cannot always be identified and in some cases may be multifactorial. In our series of 12 patients, two groups are clearly defined: those with HPV-related lesions, and those with radiation-induced lesions. Still others have no distinct precursor lesion, and further investigation may be necessary. The carcinogenic role of certain subtypes of human papilloma virus (HPV) has been well established for genital lesions.[4,5] The presence of viral antigen, viral genome, and verrucous features in some of our patients' lesions also suggest HPV as a possible causative agent in nail bed SCC.

METHODS

A nail-bed biopsy, distal phalanx amputation specimen, or both, were available from 12 reported cases of SCC (Table 1). Biopsy slides from the uterine cervix lesion were also available for case 10. All the specimens were fixed in 4% formaldehyde. Paraffin-embedded tissue was sectioned and studied by light and, in case 12, by electron microscopy. Immunohistology and *in situ* hybridization were performed as follows.

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TABLE 1. Clinical data of 12 patients with SCC

Case	Age	Sex	Location	Precursor lesion	Pathology	Follow-up
1	74	F	Lt 3	Radiodermatitis	SCC, invasive, keratinizing Other Lt hand biopsies with dysplasia	Multiple ulcers and infections both hands No tumour recurrence (7 years)
2	63	F	Rt 4	Radiodermatitis	SCC, *in situ*	Amputation, no follow-up
3	52	M	Lt 5	Verruca vulgaris C, H, IH	SCC, Microinvasive, Bowenoid Next to verruca vulgaris Amyloid deposit	Amputation, no recurrence (3 years)
4	50	F	Lt 1, 3, 4, 5	Radiodermatitis	SCC *in situ*	Local recurrence × 4 in different fingers (10 years)
5	45	F	Lt 1	Probable verruca vulgaris C, H	SCC, invasive, keratoacanthomatous Features of verruca vulgaris	Excisional biopsy No follow-up
6	66	M	Rt 1	—	SCC *in situ*, Bowenoid Warty features	Lesion incompletely excised No change (5-year follow-up)
7	57	M	Lt 1	—	SCC *in situ*, Bowenoid Warty features	No recurrence (5 years)
8	59	M	Rt 5	—	SCC, Microinvasive, Bowenoid	No recurrence (5 years)
9	81	M	Lt 1	—	SCC, Microinvasive, Bowenoid SCC, invasive, keratinizing (recurrence) Warty features	Local recurrence after 3 years No recurrence 3 years later
10	69	F	Lt 3	Verruca vulgaris C, H, IH, ISH	SCC *in situ*, Bowenoid Verruca vulgaris Uterine cervical carcinoma	Local recurrence × 3 (4 year) Phalanx amputation (recent)
11	44	M	Lt 2	? Verruca vulgaris in Rt 4, Lt 1 fgs.	SCC *in situ*, Bowenoid Warty features	Phalanx amputation No recurrence (5 years)
12	60	F	Lt 3	Verruca vulgaris C, H, IH	SCC, invasive, keratinizing Verruca vulgaris Paronychia	Local recurrence at 3 and 7 years Chronic paronychia Phalanx amputation (recent)

Positive findings by: C, Clinical; H, Histology; IH, Immunohistochemistry; ISH, *in situ* hybridization.

Immunoperoxidase

Paraffin was removed from 5 μm thick sections using xylene and graded alcohols. Endogenous peroxidase was blocked by immersing the slides in a solution of 2% hydrogen peroxide in absolute methanol. Non-specific staining was blocked with normal goat serum prior to incubation with the primary antibody. Sections were incubated with approximately 20 μl of diluted polyclonal anti-HPV rabbit serum (Dako, Santa Barbara, CA, U.S.A.) for 30 min in a humidified carrier. The sections were then washed in modified phosphate-buffered saline (PBS) and incubated with biotinylated affinity-purified goat anti-rabbit IgG (Vector, Burlingame, CA, U.S.A.) for 30 min. The sections were then incubated with avidin-biotinylated peroxidase complex (Elite ABC, Vector) for 30 min, with modified PBS washings before and after treatment. Three-amino-9-ethylcarbazole (Sigma Isopak, St Louis, MO, U.S.A.) was used as the chromagen in the presence of hydrogen peroxide. The sections were then washed in tap water, counterstained with Lerner's haematoxylin, and mounted in Crystalmount (Biomedia).[6]

In situ hybridization

Paraffin was removed from tissue sections placed on poly-l-lysine-coated slides and exposed to protease for 10 min. The slides were then washed briefly in distilled water and immersed in BLOTTO (bovine lacto transfer technique optimizer, 5% non-fat dry milk, 0·01% anti-foam A (Sigma), 0·001% thymersol) for 30 min to block non-specific DNA binding. Endogenous peroxidase was then blocked using 10% H_2O_2 in methanol. The slides were then rinsed in distilled water, dipped in graded alcohols, and air-dried. The tissue DNA was denatured by incubation at 100°C for 2·5 min in the presence of the HPV probes. Hybridization was then carried out at room temperature for 2–2·5 h. Double-stranded, nick-translated, biotin-labelled DNA probes, complimentary to the HPV genome, were used. The three probe solutions have specificities for HPV subtypes 6 and 11, HPV subtypes 16 and 18, and HPV subtypes 31, 33 and 35, respectively. A probe with human DNA genomic sequence was used as the positive control. The negative DNA control probe was specific for the plasmid vector PBR 322 (Life Technologies, Gaithersburg, MD, U.S.A.). The slides were then washed briefly with PBS–EDTA and then incubated in fresh pre-formed avidin-biotinylated peroxidase complex (Vector) for 10 min. Hybridization was detected in the same manner as previously described for immunohistological study.[7,8]

RESULTS

Clinical data

Our study included six men and six women aged 44–81 years (mean 60 years) at presentation (Table 1). All the lesions were located in the nail bed, nine on the right hand and three on the left

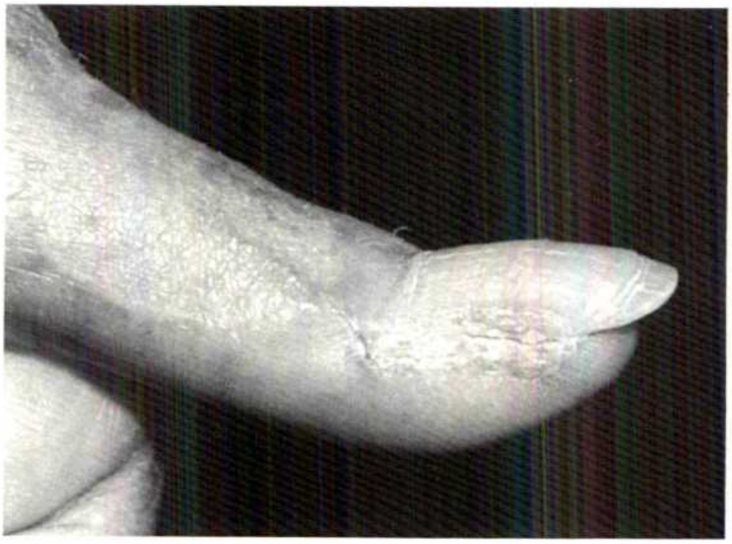

FIGURE 1. Case 4. Chronic radiodermatitis. The nail-bed tumour can be seen extending to the lateral nail fold.

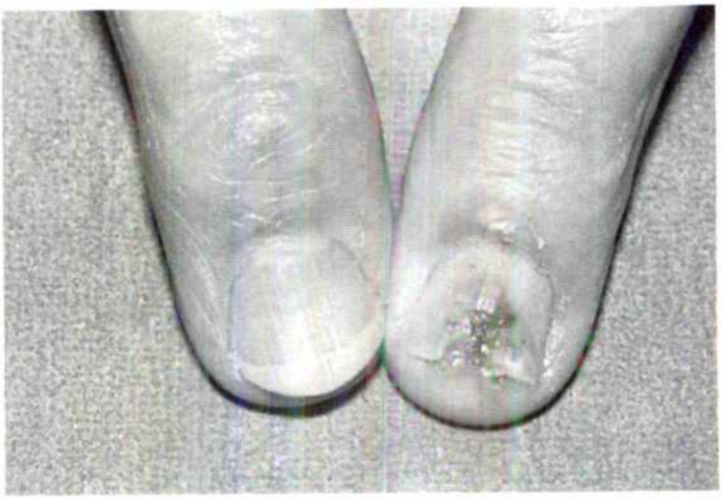

FIGURE 2. Case 12. Onycholysis and ulceration in the tumour site; paronychia is also seen in the proximal nail fold.

hand. All patients had a single lesion except Case 4, who had lesions on four fingers (first, third, fourth and fifth). The thumb was most commonly involved, being affected in five patients; the middle finger was affected in four patients (Table 1).

Three of the patients had a history of radiation exposure with a chronic radiodermatitis (Cases 1, 2 and 4) (Fig. 1), and two of these patients had biopsy-proven epithelial dysplasia or carcinoma *in situ* at other locations on the affected hand (Cases 1 and 4). A clinical history of

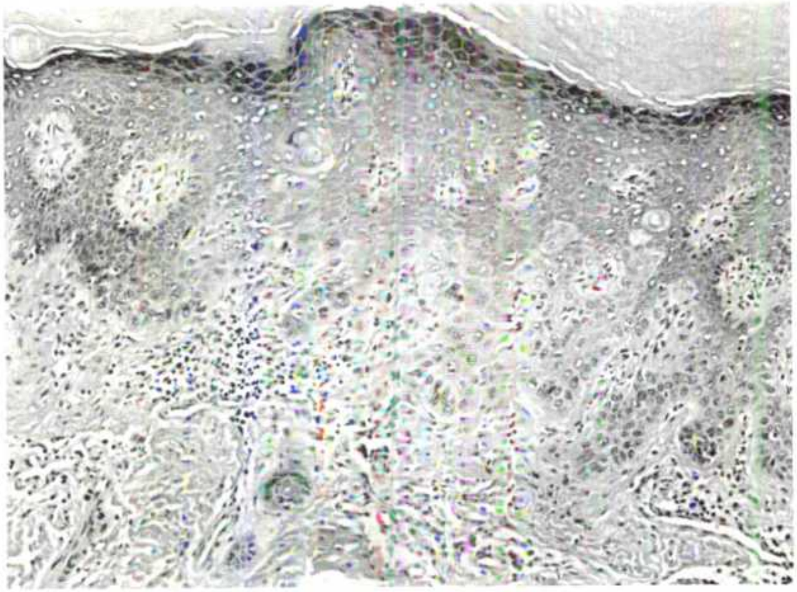

FIGURE 3. Case 8. Bowenoid SCC with foci of microinvasion (H&E × 100).

verruca vulgaris in or near the involved area was obtained in four patients (Cases 3, 5, 10 and 12). Another patient had multiple warts on other fingers as demonstrated by biopsy (Case 11).

Four of the nine patients who were treated conservatively without an amputation had local recurrences (Cases 4, 9, 10 and 12). None of the patients had any bone involvement or metastases. Paronychia with chronic inflammation was a significant clinical finding in two patients (Cases 1 and 12) (Fig. 2).

Histopathology

Biopsy specimens and/or amputation specimens were available from the 12 cases. Squamous cell carcinoma was seen in nine patients at presentation, and in three of these there were foci of microinvasion (Cases 3, 8 and 9) (Fig. 3). Patient 9 developed an invasive, keratinizing tumour 3 years after presentation. The other three patients had a well-differentiated invasive squamous cell carcinoma, and one of them displayed features of keratoacanthoma with abundant glassy cytoplasm and central foci of keratin deposition (Case 5). Features of verruca vulgaris, including papillomatosis with prominent vasculature, prominent keratohyalin granules, large parakeratotic cells, and koilocytes were seen in four cases (Cases 3, 5, 10 and 12). Focal changes suggestive of a verrucous lesion were seen in another four cases.

Amyloid deposition was prominent in the upper dermis in Case 3. Three cases (Cases 7, 9 and 11) had a marked lymphohistiocytic infiltrate with plasma cells. Acute and chronic inflammation in the upper dermis was prominent in Case 12. Special stains for micro-organisms were all negative. Hyalinized thickened blood vessels, prominent reactive fibroblasts, and dense fibrous stroma consistent with radiation effect were seen in three cases (Cases 1, 2 and 4) (Fig. 4).

Immunohistology for HPV showed focal positivity in the nuclei in three cases (Cases 3, 10 and 12) and in one the koilocytotic cells also showed strong staining within the cytoplasm (Case 12). Further study with electron microscopy demonstrated occasional non-diagnostic viral-like

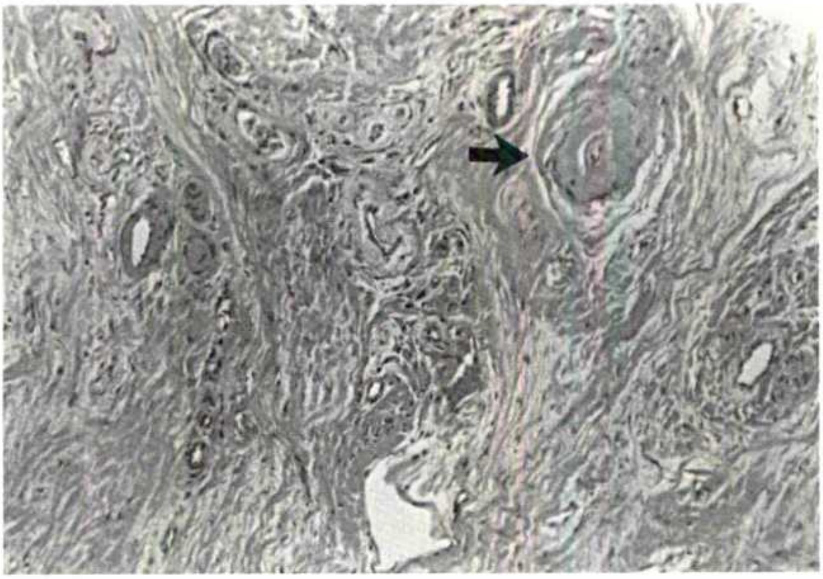

FIGURE 4. Case 4. Stromal changes of radiation damage with hyalinized blood vessels (arrow), desmoplasia and reactive fibroblasts (H&E × 200).

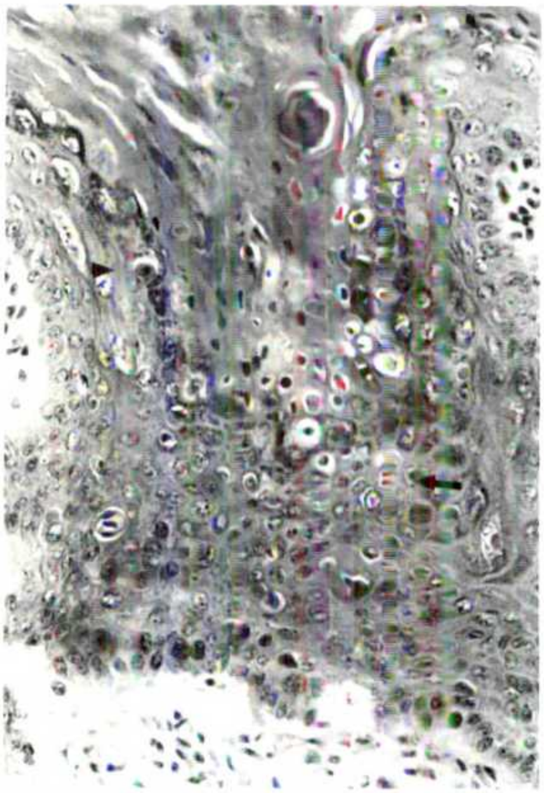

FIGURE 5. Case 10. Bowenoid SCC with hyperchromasia, pleomorphism and disarray of keratinocytes, mitotic figures (arrow) and single apoptotic cells (arrowhead) can be seen (H&E × 400).

particles. Only one out of nine cases tested with HPV probes demonstrated positive hybridization, with the probe corresponding to HPV 16 and 18 (Case 10) (Fig. 5). A uterine cervical biopsy specimen was reviewed from the same patient (Case 10) which showed a poorly-differentiated invasive SCC. Hybridization with HPV 16/18 probe was demonstrated in the tumour cells in this specimen.

DISCUSSION

The presenting symptoms of squamous cell carcinoma (SCC) of the nail bed include paronychia, ingrown nail, nail separation, nail deformity, dyschromia of the nail plate, bleeding and pain.[9–11] Invasion should be suspected when bleeding, nodularity or ulceration occur.[12] Often the diagnostic biopsy is delayed because of patients' reluctance, technical difficulties,[13] or lack of awareness by the physician and treatment is given for other suspected conditions, especially fungal or bacterial infections and for warts.[1,14]

An erythematous plaque, often with friable granulation-like tissue, or a verrucous hyperkeratotic plaque is often seen at presentation.[15] Bone involvement is seen in less than one in five of the patients[15,16] and metastases are even rarer. Two of the few reported cases with metastases were patients with hereditary ectodermal dysplasia,[1] raising the possibility of multiple primary lesions. In most cases only one digit is involved, the thumb being the most common.[10,17]

Although multiple nail-bed lesions are more commonly benign conditions,[11] patients with chronic radiodermatitis can have more than one carcinomatous lesion and close follow-up should be recommended (Case 4).[18] Chronic radiodermatitis can be a work-related disability, especially in physicians and dentists, and also an iatrogenic consequence of radiation therapy. The latter was observed in three of our patients. No distinct histopathologic findings were found in their tumours.

Verrucous lesions are often considered in the clinical differential diagnosis.[14,15] We have demonstrated the presence of papillomavirus structural proteins by immunohistochemistry, and viral genome by *in situ* hybridization. The oncogenic potential of HPV has been well established.[4] HPV DNA is detected in up to 90% of uterine cervical neoplasias, vulvar intraepithelial neoplasias, and penile carcinoma.[19] Other HPV-induced lesions with potential malignant transformation are laryngeal papillomas[20] and epidermodysplasia verruciformis.[21] Despite unsuccessful cell culture of viruses, more than 50 types of HPV have been described to date, according to their DNA homologies. A new type of HPV was recently found in a lesion of Bowen's disease of the skin.[22] HPV undergoes vegetative replication only in the superficial differentiated keratinocytes of epithelia, which are rich in keratin filaments.[19] The mechanism of inoculation of HPV into the nail matrix is incompletely understood, but successful HPV infection apparently requires direct access of the virus to the basal cells.[19] Trauma could, therefore, be listed as a predisposing,[23,24] or even a required condition and viral proliferation may then depend on activation of cell division during healing. Conversely, the dysplastic epithelium may simply provide fertile cellular conditions for viral replication. Various sized fragments of episomal and integrated viral DNA have been found in SCC *in situ* of the finger. Ostrow *et al.* suggest that polyclonality may represent several stages of viral development within the primary tumour.[25]

The presence of HPV DNA in subungual SCC may be as common as in cervical cancer. HPV genome was found in eight out of 10 periungual lesions by dot-blot analysis of frozen tissue and six of them were related to HPV 16.[26] The technique applied for DNA hybridization from paraffin-embedded sections may not be as sensitive as dot-blot analysis on frozen tissue. We noticed that positive staining by *in situ* hybridization and immunohistochemistry was restricted to focal areas, and in some cases only a few cells demonstrated definitive nuclear staining. Viral particles are known to disappear as lesions age or become malignant.[27] Therefore, we suspect a high false-negative rate due to sampling errors, limited biopsy tissue, or an advanced malignant process with possible deletion of viral markers. None the less, we found positive hybridization with HPV 16/18 probe in one out of nine patients tested. The presence of the same HPV subtypes in the genital, as well as in the nail bed, lesions on one of our patients (Case 10) could be a coincidence or could be explained by direct contact. The latter hypothesis could explain the more common presence of these lesions in the upper extremity.

Recently, a patient was described with HPV-35 positive Bowenoid papulosis in the anogenital region, who 11 years later developed a verruca with Bowenoid dysplasia and the same HPV type on her right ring finger.[28] It was suggested that scratching may have resulted in auto-inoculation. The same study found a higher viral DNA content in the finger than in the vulvar lesion. The virus probably plays an active role in keratinocyte proliferation and dysplastic changes in the early veruccal periungual lesion. The chronic vulvar lesions, like the advanced cervical carcinoma in our study, may proliferate independently of the viral genome.

In conclusion, our study supports that both radiodermatitis and HPV-infection are the most common factors for the development of SCC of the nail bed. A thorough search for possible anogenital lesions may be indicated in patients with periungual or subungual verrucal lesions.

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Female predominance of immune response to SSA/Ro antigens and risk of neonatal lupus erythematosus

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SUMMARY

Eight-hundred and twenty-five patients attending the hospital with clinical signs and symptoms of collagen diseases were tested for anti-SSA/Ro antibody. The frequency of positivity to SSA/Ro was significantly higher in females, 11·0%, than in males, 3·2% ($P < 0{\cdot}01$). Five babies born to anti-SSA/Ro positive mothers all had neonatal lupus erythematosus and in one there were various cardiac abnormalities.

There is considerable evidence that the sex of an individual has a pronounced effect on the immune system. Many autoimmune disorders are predominant in females, particularly systemic lupus erythematosus (SLE).[1] Surveys of large groups have indicated a greater prevalence of anti-nuclear antibodies in females than males[2] and anti-SSA/Ro is detectable in 25–30% of patients with SLE.[3] These patients have a particular subset of SLE, subacute cutaneous lupus erythematosus.[4] Anti-SSA/Ro is an antibody that can be transferred from the mother to the infant and can result in neonatal lupus with congenital heart block.[5-9] The present study was carried out to determine whether the response to SSA/Ro antigens varies according to sex, and to evaluate the risk of neonatal lupus erythematosus in those infants born to SSA/Ro positive mothers.

METHODS

Serum was obtained from 825 patients with signs and symptoms of collagen diseases. The presence of antibodies against SSA/Ro and SSB/La were determined by double immunodiffusion. Extracts of rabbit thymus and human spleen were used as the source of antigens as previously described.[10] Antinuclear antibodies were detected by indirect immunofluorescence with HEp-2 cells which served as a tissue substrate. The sera from normal healthy volunteers

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were used for the negative controls. The reference sera against SSA/Ro and SSB/La were provided by the ANA Reference Laboratory (Immunology Branch, Center for Disease Control, Atlanta, GA, U.S.A.).

RESULTS

Of 825 patients screened for anti-SSA/Ro antibody, 79 (9·6%) were found to be positive. The frequency of positivity to SSA/Ro antigens was higher in females (74/670, 11·0%) than in males (5/155, 3·2%). These findings were significant ($P < 0{\cdot}01$, by χ^2). All but three of the anti-SSA/Ro positive patients had anti-SSB/La antibody. The clinical diagnoses of 54 anti-SSA/Ro positive patients are shown in Table 1.

The age distribution and sex of the patients positive for anti-SSA/Ro antibody are shown in Table 2. In this group there were 30 women of child bearing age. Pregnancies occurred in four of these patients with anti-SSA/Ro resulting in five deliveries. All except one were full-term deliveries and the infants had normal birth weights. Three had cutaneous lesions of lupus. None of the infants had congenital heart block, but in one there were a number of cardiac abnormalities as have been previously described[11] (Table 3). None of the mothers had any exacerbation of their SLE during pregnancy and in the puerperium.

Cases 1 and 2

These were male infants born to a mother with anti-SSA/Ro and SSB/La positive SLE. They developed annular erythema on the face and extremities 5–7 weeks after birth. The lesions resolved before the age of 4 months, and at that time anti-SSA/Ro and SSB/La were no longer detectable. These patients have been described in more detail elsewhere.[6]

TABLE 1. Clinical diagnosis of anti-SSA/Ro positive patients at follow up

Diagnosis	Number of cases
SLE	22
PSS	3
Polymyositis/dermatomyositis	2
Overlap PSS-polymyositis	1
SLE-dermatomyositis	1
Primary Sjögren's syndrome	10
Rheumatoid arthritis	3
Drug eruption	2
Others*	10
Total	54

*Others include Raynaud's phenomenon, swollen hands, cutaneous vasculitis, subclinical Sjögren's syndrome, photosensitive dermatitis, discoid lupus erythematosus, and febrile episodes with arthralgias

TABLE 2. Age and sex distribution of anti-SSA/Ro positive patients

Age	Male	Female
11–19	—	4
20–29	—	4
30–39	2	14
40–49	—	15
50–59	1	5
60 and above	—	9
Total	3	51
Average (years)	42·0	42·1

Case 3

This child was born to a 22-year-old healthy woman and at that time showed no abnormality. At the age of 10 days, an annular erythematous rash developed on the forehead followed by other lesions on the face, trunk and extremities. All these lesions disappeared within 12 weeks. Laboratory investigations at the age of 3 weeks showed elevated levels of liver enzymes. There were positive fluorescent antinuclear antibodies, anti-SSA/Ro and anti-SSB/La antibodies in titres of 1:640, 1:40 and 1:20, respectively. These became negative at the age of 6 months. Serological tests for anti-native DNA, antinuclear RNP and anti-Sm antibodies were negative. There was no hepatosplenomegaly and the child was not jaundiced.

Although the mother did not meet the criteria for SLE or other collagen disorders, she had hypergammaglobulinaemia, positive fluorescent antinuclear antibodies, positive anti-SSA/Ro and SSB/La antibodies and an abnormal sialogram.

Case 4

A male infant was born prematurely and developed a fever associated with a cough and convulsions. There were no cutaneous lesions or hepatosplenomegaly. Laboratory investigations indicated elevated levels of liver enzymes. The fluorescent antinuclear antibody, anti-SSA/Ro and anti-SSB/La were positive at titres of 1:40, 1:1 and 1:1, respectively. These became negative when the child was 4 months old. An electrocardiogram was normal.

The 32-year-old mother of this infant had a 5-year history of SLE manifested by recurrent febrile episodes with arthralgia and an occasional malar rash and also lymphadenopathy. She

TABLE 3. Neonatal complications in infants of mothers with anti-SSA/Ro autoantibody

Patient no.	Sex	Gestational age (weeks)	Weight (g)	Complications	Maternal features
1[6]	M	38	2800	Skin lesions	SLE, no medication
2[6]	M	40	2980	Skin lesions	
3	F	40	3000	Skin lesions and hepatitis	Asymptomatic
4	M	37	2350	Hepatitis	SLE, no medication
5[11]	M	40	3400	Cardiac abnormalities	Raynaud's phenomenon, no medication

had an elevated ESR, occasional leukopenia, hypergammaglobulinaemia and positive fluorescent antinuclear antibody, positive anti-native DNA and positive anti-SSA/Ro and SSB/La antibodies. During the pregnancy, which was her first, she remained well.

Case 5

This infant presented with a number of cardiac abnormalities that included an interruption of the aortic arch, patent ductus arteriosus, ventricular septal defect and double-outlet of the right ventricle. The child's mother had Raynaud's phenomenon and was anti-SSA/Ro positive. This case has been described elsewhere.[11]

DISCUSSION

In this study we found a predominance in females of SSA/Ro antibodies and confirmed their association with neonatal lupus. This disorder affects newborn infants of mothers who are anti-SSA/Ro positive and who also, more rarely, have antinuclear RNP.[12,13] Anti-SSA/Ro antibody has been found in the serum of over 95% of mothers of infants with the neonatal lupus syndrome.[12] The infants usually have complete heart block and/or a lupus rash, and they may have hepatosplenomegaly, pneumonitis and various haematological abnormalities. Hepatitis has occurred in association with congenital heart block and/or a lupus rash in this condition.[1,5,7,14,15] Three infants were recently reported with cholestatic jaundice and hepatitis as major features of neonatal lupus erythematosus.[16] These reports and our cases indicate that hepatitis is one of the manifestations of the neonatal lupus syndrome.

None of our patients had any complications during their pregnancies. The four anti-SSA/Ro-positive women gave birth to five infants. The prevalence of spontaneous abortion (1/7) did not differ significantly from other published data.[15,17] According to Watson *et al.*[12,18] the rate of spontaneous abortion or stillbirth in the mothers of 10 cases of neonatal lupus erythematosus was 22%, and was 28% in 34 anti-SSA/Ro positive women. Although these studies have not noted the importance of congenital heart block or cardiac abnormalities in the foetal deaths, anti-SSA/Ro antibody may be associated with the cardiac damage *in utero*.[11,19,20]

Previous studies for anti-SSA/Ro antibody in normal mother and infant pairs[8] and on 71 infants from birth to 5 months of age for both anti-SSA and SSB antibodies[21] were found to be all negative. However, 1% of pregnant women[22] and mother/infant pairs[23] were found to be positive for anti-SSA/Ro but otherwise showed no evidence of autoimmune disease or neonatal lupus. Mothers of infants with neonatal lupus are often asymptomatic,[15,24] but later many develop the symptoms of collagen disorders.[15]

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The treatment of port wine stains with the carbon dioxide laser

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SUMMARY

The carbon dioxide (CO_2) laser was used to treat 51 patients with port wine stains (PWS). Twenty-nine patients were adults who had failed to respond to argon or continuous wave dye laser therapy and 22 patients were children with pink PWS. Follow-up assessment of 40 patients 12 months after treatment revealed an excellent or good result in 74% of adults and 53% of children. The results in adult 'therapeutic failures' was encouraging and CO_2 laser therapy can be advised for this group. Two children had a poor result, one with a hypertrophic scar, and treatment in this age group with the CO_2 laser should be considered with caution.

The argon laser has been used to treat port wine stains (PWS) for over 10 years. A good or excellent response to treatment can be expected in 60–80% of patients, the best results being seen in those adults with purple port wine stains. The incidence of scarring with this laser varies from 9–23%.[1–3] In children who have predominantly pink PWS, results are less satisfactory, with less than 50% benefitting from treatment[1,2,4] and a 38% incidence of scarring.[5] Experience with the continuous wave dye laser emitting at 577 nm reveals similar results to the argon laser, with older patients with purple PWS achieving better responses than younger patients with pink PWS.[6,7] Preliminary results with a pulsed dye laser emitting ms pulses have suggested superior results in pink PWS.[8] However, experience with this laser is limited and both the capital costs of this equipment and its running costs are extremely high. Therefore, 20–40% of patients treated with argon or continuous wave dye laser will fail to achieve a satisfactory result and some may scar from therapy. A poor result is even more likely in chldren with pink PWS.

The CO_2 laser has also been used in the treatment of PWS, with 45–60% of patients achieving satisfactory results and an incidence of scarring of 4–8%.[9,10] This laser emits in the infra-red at 10,600 nm and is absorbed by tissue water so that the colour of the naevus should not influence outcome. While success rates are lower with this laser, the incidence of scarring is less than that of the argon laser.

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The aims of this study were to evaluate the effects of the CO_2 laser in the treatment of port wine stains in two selected groups of patients. The first group were those adults with PWS who had failed to respond to either argon or dye laser treatment. The second group were children with pink and easily compressible PWS which would not be expected to respond well to either of the other lasers available.

METHODS

Patients

Twenty-nine adults with PWS were treated with the CO_2 laser (Table 1). All these patients had failed to obtain adequate paling of their naevus after either argon or dye laser at 577 nm. Any patient who had experienced scarring with either laser was excluded from the study.

In general it is our policy to defer laser treatment of PWS in children until a therapeutic response from argon/dye laser therapy can be expected (14–16 years old). However, occasionally children are seen where the patients or the patients' parents are suffering from such distress because of the birthmark that they are unwilling to wait for argon or dye laser therapy. Twenty-two such children were offered treatment with the CO_2 laser (Table 1).

All treatments in both groups were performed on naevi on the head and neck.

Treatment

Informed consent was obtained in each case and in the treatment of children, parents were informed of possible improved results using other lasers at a later date. Seventy treatments were performed on the 51 patients. Of these, 57 were under general anaesthesia and all the children received treatment in this way. Thirteen treatments were performed under local anaesthesia, using plain lignocaine and this was usual for the initial test treatments in adults.

In all patients an initial test treatment was performed on an area 1–4 cm in diameter, and where possible in an inconspicuous part of the birthmark. Areas near the eyes were avoided because of the risk of damage to the eyes from the laser beam.

Treatment was at low powers of 5–10 W using a defocused beam with a spot diameter of 3–5 mm (energy fluence 50–140 W/cm^2) in a continuous mode. An airbrush technique was used to induce a superficial epidermal blister which was then removed to expose the ectatic dermal vessels. Further passes of the laser beam (usually 2–4) were performed to coagulate these vessels.

The treatment area was left exposed where possible. If not, a dressing with paraffin gauze was

TABLE 1. Details of patients treated

Adult 'failures'		Children
No. 29 (18 female, 11 male)		22 (15 female, 7 male)
Mean age 33·1 years (16–61)		10·1 years (1–14)
Predominant colour:		
Purple	11	All pink and easily blanchable with pressure
Pink/purple	4	
Red/purple	2	
Red	10	
Pink/red	2	

applied. The exposed wound rapidly developed a firm crust overlying the treated area which slowly separated over 2–3 weeks.

Patients were reviewed 1 week after treatment to assess wound healing and at 6 or 12 months to assess response to therapy. An excellent result was normal or near normal skin colour without scarring or pigmentary change. A good result showed significant lightening without scarring or hyperpigmentation. A fair result showed slight pallor of the naevus with or without atrophic scarring but without hyperpigmentation or hypertrophic scarring. A poor result was a hypertrophic scar or hyperpigmentation. These assessments were the same as those employed in assessing treatment of port wine stains with the tunable dye laser.[7]

RESULTS

Histology

In an attempt to correlate histological changes with treatment variables and outcome from therapy, histological examination of the superficial blister raised after the initial first laser pass was performed in 22 patients and a 3 mm punch biopsy from the area at the end of the treatment was performed in 10 selected patients.

The blister in all cases consisted only of epidemis which was of full or partial thickness. Occasional thermal damage was seen in the deep epidermis, and a prominent feature was follicular keratotic plugging. These changes were unrelated to the power outputs used and were not specific to PWS, being seen after treatment to tattoos[11] and a number of other dermatological conditions.

The changes seen after laser treatment were absence of the epidermis with occasional superficial thermal damage to the dermis as a homogenized band. Skin appendages were in general spared, and occasional vascular channels could be seen in the deeper dermis which may have been residual ectatic vessels of the PWS. The presence of thermal damage did not correlate with eventual outcome from therapy, and was unrelated to the powers used.

Results of treatment

Forty of the 51 patients (17 children, 23 adults) have been followed up for 12 months or longer, and responses to treatment have been assessed in this group. Assessments were based on the global area treated which in 27 patients was the initial test area, and in 13 patients was based on a total of 32 treatments.

The results of treatment are shown in Table 2. Among the 17 adults who had a good or excellent result, eight were females. The mean age of this group (28·5 years) was lower than the

TABLE 2. Assessment after treatment (12 months or longer)

	Adults $n = 23$ (%)	Children $n = 17$ (%)
Excellent or good	17 (74)	9 (53)
Fair	4 (17)	4 (23)
Poor	0	2 (12)
No effect	2 (9)	2 (12)

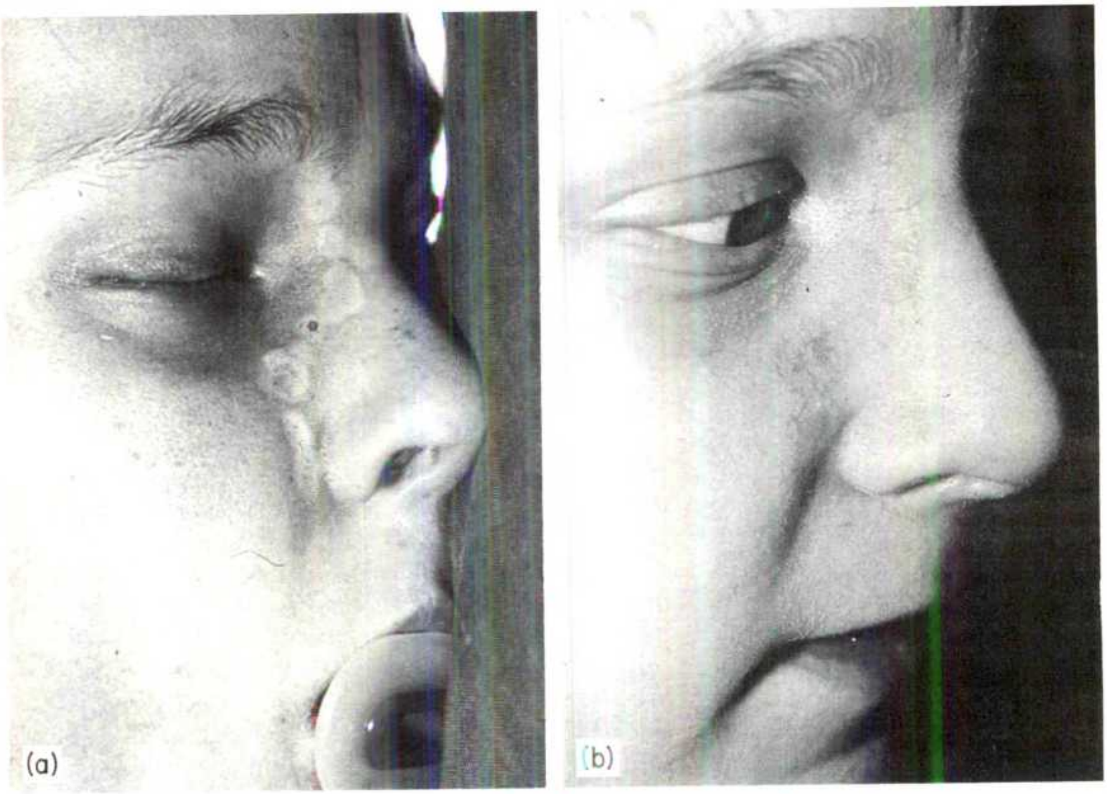

FIGURE 1. (a) PWS on side of nose immediately after laser therapy. (b) Excellent result from treatment to PWS.

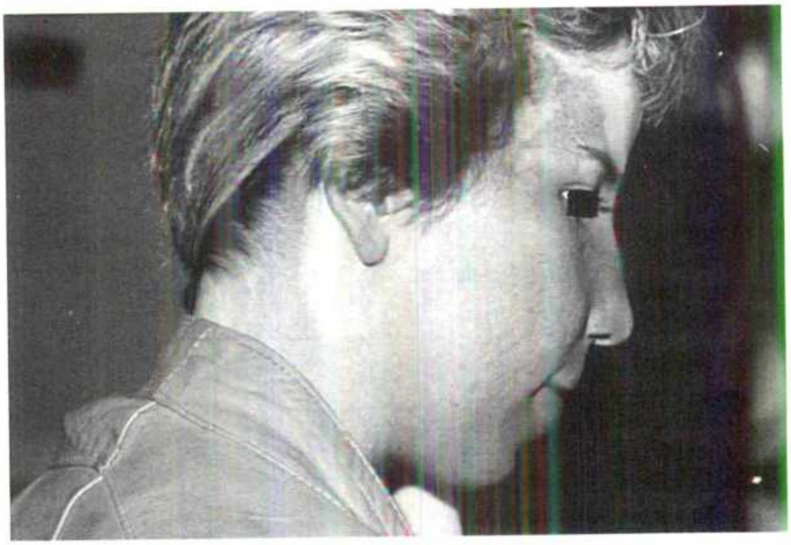

FIGURE 2. Excellent results after treatment around ear.

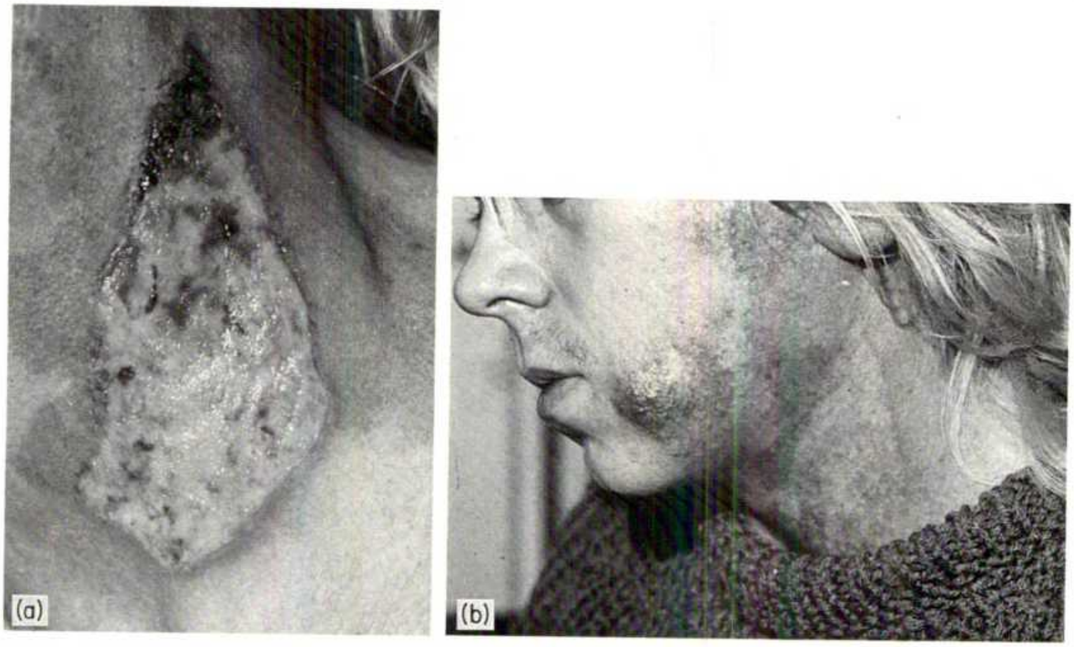

FIGURE 3. (a) PWS on side of neck one week after treatment. (b) Good result to treated area.

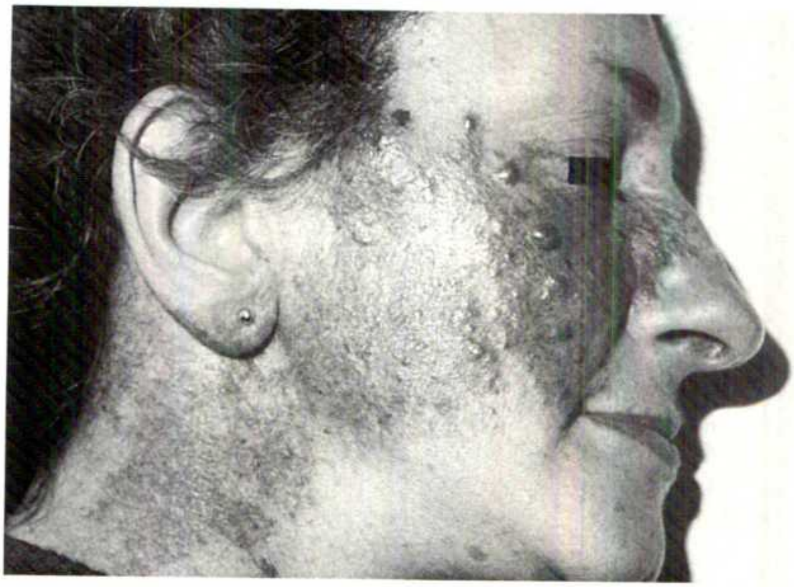

FIGURE 4. Good result after treatment of lateral areas of PWS on cheek and neck.

original treatment group. There was no predominant colour of naevus in those who responded well. No adult experienced a poor result. Representative results are illustrated in Figures 1–4.

Two children had poor results; one developed a hypertrophic scar on the neck, the other developed marginal hyperpigmentation in an otherwise satisfactory test area. Neither event was predictable from the treatment variables used and wound healing had proceeded in a similar manner to other patients.

In four patients, the laser produced no discernible change in the PWS when assessed at 12 months. Morbidity from treatment was low. There were no wound infections and discomfort was mild, although one adult female complained of severe pruritus at the site of treatment which persisted for 3 weeks after the wound had healed.

DISCUSSION

The results from this study show that 74% of adults and 53% of children assessed after CO_2 laser treatment of their PWS have obtained an excellent or good result. These results are very encouraging, as children with pink PWS respond poorly to argon and continuous wave dye lasers[1,2,4,5] and the adult population in this study was selected after having failed to respond to prior treatment with these lasers.

The CO_2 laser emits in the infra-red at 10,600 nm. Light at this wavelength is absorbed by water and there is therefore no selectivity of absorption in the tissue. This would explain why the colour of the naevus is less important in influencing therapeutic outcome when using the CO_2 laser.

The techniques used in this study of low power and irradiance did result in some thermal damage to residual tissue, although this did not inevitably lead to scarring. Previous histological studies of CO_2 laser treatment of PWS[12,13] have revealed considerable thermal damage to the dermis with necrosis seen most clearly around the blood vessels. These results contrast with the commonly reported ability of the CO_2 laser to vapourize tissue with minimal injury to adjacent structures. It has been calculated that long pulses of low power densities with this laser produce deeper thermal damage than short pulses of high power intensity,[14] and the development of super-pulsed lasers with short pulse durations should avoid this problem.[15]

The pulse durations used with the argon laser also result in a non-specific thermal effect from heat radiation and this damage to dermal connective tissue may be necessary for a satisfactory result. In contrast, flashlamp-pulsed tunable dye lasers using wavelengths of 577 nm and 360 μs pulses produce specific vascular damage with sparing of the dermal connective tissue.[13,16] This laser has been reported to produce satisfactory pallor in children with PWS,[8] but experience with this laser is limited to only a few centres world-wide.

Two (12%) children developed adverse reactions to treatment, one of which was a hypertrophic scar. However, 53% of children treated had an excellent or good response, and two children showed no response to the laser and would be suitable for retreatment. Treatment of PWS in children should proceed cautiously no matter which laser is used, as they appear to have an innate tendency to develop hypertrophic scarring.[5] It may be preferable to defer treatment until a time when the risk of scarring becomes less likely. If treatment is requested, until further experience with pulsed dye lasers becomes available, a trial treatment with the CO_2 laser should be considered.

The results in adult patients are comparable to the best results with the argon laser. The CO_2 laser is much more widely available than the argon laser and quicker to use. This laser, however, can produce severe cutaneous damage and although its use in the treatment of adults with port wine stains can be recommended, this should be under the direct supervision of a clinician experienced in laser use and safety.

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Cutaneous malignant melanomas occurring under cyclosporin A therapy: a report of two cases

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SUMMARY

Two patients are reported with cutaneous malignant melanoma who had been on treatment with cyclosporin A. The first case was a 44-year-old man with systemic sclerosis and the second a 52-year-old woman who had a renal transplant. In both cases cyclosporin A was administered with a low dose of prednisone.

Cyclosporin A (CyA) is a cyclic undecapeptide of fungal origin which has been shown to have immunosuppressive properties without toxic effects on the bone marrow.[1] The mode of action of CyA is complex and only partially understood,[1,2] but CyA inhibits the release of interleukin 2 and other lymphokines by activated T lymphocytes and the response of precursor cytotoxic lymphocytes to interleukin 2. It also inhibits lymphokines which affect macrophages. Cyclosporin has been used in the treatment of a variety of skin disorders.[2–5] We report two patients who developed cutaneous malignant melanomas while on CyA therapy for 20 and 19 months, respectively.

CASE REPORTS

Case 1

In the autumn of 1985, a 44-year-old man had paraesthesia affecting the tips of the fingers which was followed by swelling and stiffening of the hands. He then observed a progressive tightening of the skin of the arms and the trunk. A diagnosis of systemic sclerosis was made in February 1986 and confirmed by a skin biopsy. He had moderate cardiac failure and obstructive lung disease. Treatment with prednisone produced a rapid improvement of his cardiac and pulmonary symptoms. In October 1986 he was found to have a monoclonal IgG κ gammopathy. Cyclosporin A in a dosage of 4 mg/kg per day on 5 days a week was added to prednisone therapy

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and this improved the cutaneous symptoms and the monoclonal gammopathy. Therapy with CyA was maintained at the same dosage while the dose of prednisone was gradually reduced to 10–15 mg every other day. In June 1988, an irregular pigmented nodule, 8 mm in diameter was observed on his back and the lesion was excised. There was no pre-existing pigmented lesion at that site. On histology, the lesion was a superficial spreading malignant melanoma and the depth of invasion was Clark level III, with a maximum Breslow thickness of 0·8 mm. In July 1988, the area was re-excised. An extensive investigation for metastases, which included cerebral, thoracic and abdominal CT scans was negative. There were no other predisposing factors for melanoma in this patient.

Case 2

A 50-year-old woman was admitted to the hospital for a kidney transplant that was performed in August 1985. Over the previous 5 years she had been on haemodialysis for chronic renal failure that was associated with hypertension from a 30-year history of phenacetin abuse and other analgesics. Rejection of the graft was prevented by treatment with CyA and prednisone. The dose of CyA varied between 5 and 7·5 mg/kg per day during the first 2 weeks and was then gradually reduced to 1·6–1·7 mg/kg per day. She was treated with prednisone initially at 30 mg/day and the dose was gradually reduced and then stopped in June 1986.

In December 1986, the patient noticed that a brown mole on her right cheek that had been present for many years had increased in size. In January 1987 the lesion was removed and on histology was found to be a malignant melanoma of the superficial spreading type with the depth of invasion being Clark level II, and a Breslow thickness of 0·38 mm. There was no evidence of metastases on clinical examination.

DISCUSSION

To our knowledge, these are the first cases of malignant melanoma arising during therapy with CyA. Our first patient, from the onset of his systemic sclerosis, was examined by several physicians including dermatologists and none had noted a mole at the site where the melanoma developed. Furthermore, on histology there was no evidence of any residual naevus. These data favour a '*de novo*' occurrence of the melanoma while the patient was on therapy with CyA. Our second patient had a pigmented lesion for several years at the site where the malignant melanoma subsequently developed. Malignant transformation of a previously benign lesion appears to be likely in this case.

Many other cutaneous and non-cutaneous malignancies have been reported in association with therapy with CyA.[6] The average time for the appearance of tumours was after 20 months of CyA therapy[6] and this corresponds to what occurred in our two patients. The incidence of lymphomas and lymphoproliferative disorders,[6-11] some of which affect the skin, is higher with CyA than with other immunosuppressive drugs.[6] Cutaneous conditions associated with CyA therapy include bowenoid changes, actinic keratoses, basal cell and squamous cell carcinomas.[6,12-20] Their incidence varies between 2%[18] and 15%[6] of organ transplant recipients. The exacerbation of the cutaneous manifestations of a Torre's syndrome was described in a cardiac transplant patient receiving CyA.[21]

The use of CyA as a single immunosuppressive agent was reported in a model of human melanoma transplanted into rats.[22] It was demonstrated that in mice immunosuppressed with CyA the lethality of transplanted human melanoma cells was increased.[23] The influence of CyA on the host defences was studied with regard to the metastatic potential of B16F10 melanoma cells injected into syngenic mice.[24] These animal studies indicate that, at least in rodents, CyA could favour the development and spread of malignant melanomas.

Cutaneous malignant melanomas have already been reported to have a higher incidence in patients systemically or locally immunosuppressed by agents other than CyA, including in kidney transplant recipients.[25-27] The occurrence of a melanoma in patients immunosuppressed with CyA is not surprising. Although it is possible that these melanomas occurred coincidentally during therapy with CyA it is more likely that this immunosuppressive drug favoured the development of these malignancies.

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Discoid lupus erythematosus associated with hereditary angioneurotic oedema

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SUMMARY

A female patient developed discoid lupus erythematosus 6 years after the onset of hereditary angioneurotic oedema. Treatment with danazol for 18 months cleared both conditions and was well tolerated.

CASE REPORT

A 38-year-old female developed intermittent swelling of the hands lasting from 2 to 5 days during her first pregnancy in 1967 when aged 16 years. A few weeks after an uneventful delivery, stilboestrol was prescribed for suppression of lactation and precipitated attacks of abdominal pain, vomiting and carpopedal spasm. Subsequent attacks of angio-oedema affecting the face hands, gut and larynx were induced by oral contraceptives and dental extraction and some occurred spontaneously. The attacks gradually increased in frequency and severity, and there was at least one episode of life-threatening laryngeal oedema. There was no family history of angio-oedema. Erythematous scaly plaques first appeared on the forehead and cheeks in 1973 (Fig. 1), and involvement of the scalp produced a 5 cm diameter area of scarring alopecia. A diagnosis of discoid lupus erythematosus (DLE) was confirmed by a skin biopsy which showed epidermal atrophy, follicular plugging, periadnexeal inflammatory infiltrate and hydropic degeneration of the basal layer.

Serological investigation revealed a C1 esterase inhibitor level of 0·02 g/l (normal 0·18–0·26 g/l); C4 0·03 g/l (normal 0·14–0·54 g/l and IgA 0·75 g/l (normal 0·80–4·50 g/l). The following tests were either normal or negative: full blood count, erythrocyte sedimentation rate, biochemical profile, antinuclear antibody, DNA binding, rheumatoid factor, C1q, C3c, circulating immune complexes, IgG, IgM and direct immunofluorescence of non-lesional skin. Family screening for C1 esterase inhibitor and IgA deficiency in the parents and three children of the patient was also negative.

Treatment with a variety of topical corticosteroids including flurandrenolone (Haelan tape®), antimalarials and a 6-week course of oral prednisolone 20 mg daily was unsuccessful, as was a course of auranofin (Ridaura®) 6 mg daily for 10 weeks. Dapsone 75 mg daily produced

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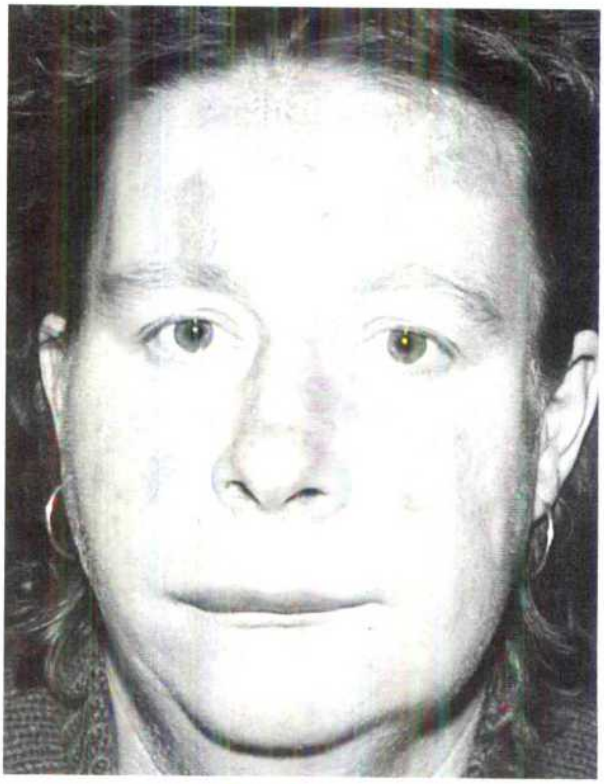

FIGURE 1. Florid DLE and angioedema of the upper lip.

headaches and was stopped after 2 months, and clofazimine 100 mg daily was discontinued after 2 months as it induced painful mouth ulcers. Thalidomide 100 mg daily produced an initial benefit but it was withdrawn after 18 months as the disease activity relapsed. Danazol 200 mg daily was commenced in 1987 and completely abolished attacks of angioedema, raised the levels of C1 inhibitor and C4 to 0·24 g/l and 0·23 g/l, respectively and markedly improved the DLE. Treatment with danazol was well tolerated over a 2-year period though the side-effects included weight gain and cessation of menses.

DISCUSSION

A diagnosis of hereditary angioneurotic oedema (HANE) in the above patient was supported by a C1 inhibitor level that was reduced to 10% of normal, and an early onset of recurrent attacks of angio-oedema affecting the face, extremities, gut and larynx. The attacks of angio-oedema gradually became more frequent over a 22-year period, but there was no evidence for the presence of any other systemic disease. In contrast, acquired C1 inhibitor deficiency usually occurs in elderly patients and is associated with an underlying lymphoproliferative disease or multiple myeloma in over 90% of cases.[1] Furthermore, an acquired C1 inhibitor deficiency occurs secondarily to a low C1 and this complement component was normal in our patient. However, HANE is inherited as an autosomal dominant trait and the absence of a family history of HANE and the normal C1 inhibitor level in the parents and three children of our patient remains unexplained.

Heterozygous conditions with an autosomal dominant mode of inheritance are characterized by a reduction in enzyme activity to 50% of normal. It is therefore interesting that the C1 inhibitor level in HANE is reduced to between 5 and 30% of normal.[2] Studies on cultured monocytes from patients with HANE have revealed an intracellular level of C1 inhibitor that is approximately 50% of normal.[3] This suggests that consumption of the protease inhibitor occurs either during or after cellular secretion.

There has been considerable speculation regarding the nature of the primary inflammatory mediator in HANE. Histamine appears unlikely to be the main mediator as antihistamines have been found to be ineffective in preventing or treating attacks of angio-oedema. Attention has recently focussed on bradykinin as C1 inhibitor can influence the synthesis of bradykinin from high molecular weight kininogen by exerting an inhibitory effect on kallikrein.[4,5] Furthermore, a raised serum level of bradykinin has been observed during attacks of angioedema.[6,7,8] However, subcutaneous injection of bradykinin produces a swelling which is erythematous and painful and intravenous bradykinin is accompanied by hypotension, and these are not features of HANE.[2]

An association between HANE and DLE was suggested by the finding of a 200-fold increase in the incidence of DLE in patients with HANE as compared with the general American population.[9] In addition, danazol, an attenuated androgen, was found to correct both disease processes simultaneously.[10] A possible pathogenic mechanism by which C1 inhibitor deficiency might induce DLE involves the early complement components. Under normal circumstances C1 is activated by a number of factors, including immune complexes. The activated components of C3 and C5 are responsible for efficient opsonization, solubilization and degradation of immune complexes. In C1 inhibitor deficiency complement activation is initially accelerated, but this rapidly depletes the early complement components such as C2 and C4, and causes the pathway to function at a suboptimal level. Circulating immune complexes are therefore no longer rapidly removed and become deposited in tissues such as the skin with subsequent development of DLE.

An association between DLE and HANE is rare, but its recognition is important as both conditions respond to danazol therapy. This treatment is well tolerated, and the side-effects are related to the anabolic and antigonadotropic properties of the drug.

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Benign cephalic histiocytosis: a case report

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SUMMARY

The case is reported of a child who presented with brownish macules and papules on the face and in whom biopsies confirmed a diagnosis of benign cephalic histiocytosis. The clinical and histological features of our case are typical for this rare self-healing non-Langerhans cell histiocytic disorder.

CASE REPORT

A 14-month-old Caucasian boy was referred with a history of an asymptomatic facial rash that had started on the eyelids and cheeks at the age of 2 months. The child was otherwise well and there was no family history of significance. On examination there were brownish macules and papules confined to the forehead, eyelids, cheeks and upper neck (Fig. 1). The appearances were somewhat similar to those of urticaria pigmentosa, but the lesions did not urticate. General examination showed no evidence of systemic disease. At the age of 4 the lesions had started to regress. Blood investigations including serum lipid analysis, liver function tests and full blood count were all normal, as were X-rays of the skull and chest.

A skin biopsy of a facial papule was performed and this showed a dense upper and mid-dermal infiltrate that extended to the epidermis, but did not invade it. The infiltrate was predominantly histiocytic with small numbers of lymphocytes and eosinophils (Fig. 2). The histiocytes had a glassy cytoplasm, and their nuclei showed sparse chromatin and prominant nucleoli. Metachromic stains showed only a few positive mast cells adjacent to the blood vessels. Using immunoperoxidase techniques, the histiocytes were negative for S-100 protein, in contrast to the positive staining of the Langerhans cells in the epidermis. A few multinucleated histiocytic cells were present, but none of the Touton form. Electron microscopy (Fig. 3) failed to demonstrate any Birbeck granules or definable comma-shaped bodies within the dermal histiocytes, but coated vesicles could be identified in the cytoplasm.

DISCUSSION

Benign cephalic histiocytosis was first described in 1971.[1] All 18 cases described in the literature had their onset between the ages of 6 months and 3 years and had started to regress

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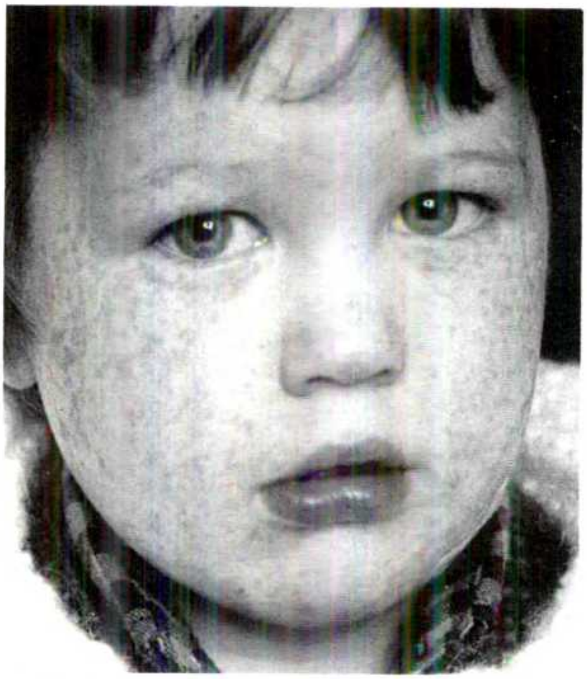

FIGURE 1. Benign cephalic histiocytosis. Numerous facial macules and papules.

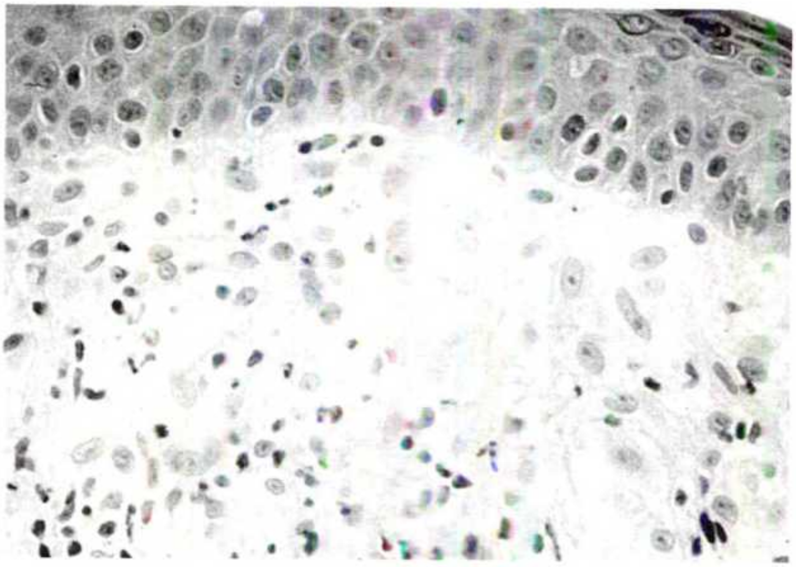

FIGURE 2. Predominantly histiocytic dermal infiltrate with a few giant cells. Smaller numbers of lymphocytes and eosinophils are intermixed. (H&E; × 280).

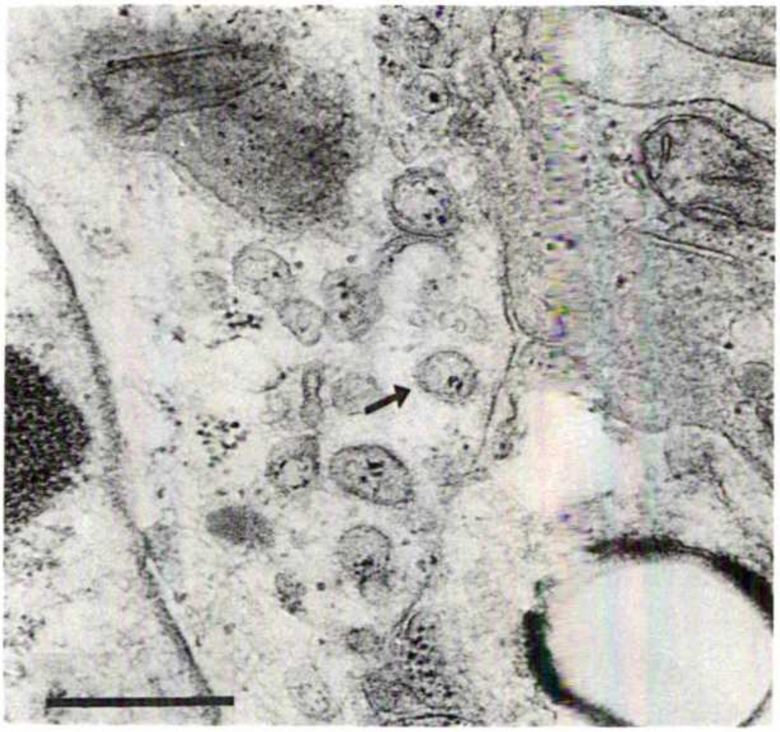

FIGURE 3. Electron micrograph of a dermal histiocyte. Arrow indicates one of several coated vesicles (× 28,000; bar 1 μ).

within 8 months to 4 years.[2-4] In all cases with sufficient follow-up the eruption healed completely without scarring within 4–9 years. The initial lesions have always been on the head, with subsequent spread to the trunk and limbs in a few cases. In no case has there been involvement of the mucous membranes or viscera.

Histologically, the histiocytic infiltrate in benign cephalic histiocytosis lacks foamy cells or Touton giant cells, and markers for Langerhans cells are negative. These histological features are similar to those of generalized eruptive histiocytoma.[5] Although some observers consider the two conditions to be very closely allied,[6] generalized eruptive histiocytoma is typically a disease of adults in which macules are absent and there is no cephalic predominance of lesions. In generalized eruptive histiocytoma the mucous membranes may be involved, although the viscera are spared as in benign cephalic histiocytosis. Ultrastructurally dense and laminated bodies are the typical finding in generalized eruptive histiocytoma, rather than the coated vesicles and comma-shaped bodies as seen in benign cephalic histiocytosis. The significance of these features is unknown. Biopsy material was not available to carry out extensive immunocytochemical investigations.

The eruption of the small nodular form of juvenile xanthogranuloma is composed of yellowish papules and nodules which histologically contain many multinucleate cells and giant cells. It is important to distinguish benign cephalic histiocytosis from Langerhans cell histiocytosis (histiocytosis X). The combination of papules on the scalp and trunk that are scaly and crusted with positive Langerhans cell markers on immunohistochemistry are distinctive for Langerhans cell histiocytosis.

We feel that in the past, cases of benign cephalic histiocytosis have been misclassified as atypical cases of juvenile xanthogranuloma or urticaria pigmentosa without being biopsied, or possibly as a benign variant of Langerhans cell histiocytosis.

ACKNOWLEDGMENT

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Disseminated porokeratosis in an infant with craniosynostosis

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SUMMARY

An infant with craniosynostosis and other congenital defects developed a progressive skin rash from the age of 1 month. Histological examination revealed dyskeratosis and a cornoid lamella suggestive of porokeratosis. This patient is remarkable for the early onset and severity of the skin disease.

Cutis aplasia[1] and epidermal naevi[2] have been described in association with craniosynostosis. There have been no previous reports of porokeratosis or any disorder of keratinization occurring in association with craniosynostosis.

CASE REPORT

The patient was a male infant born at term after a normal pregnancy and the first child of healthy unrelated parents. He was noted to have craniostenosis due to fusion of the coronal and sagittal sutures, in addition to hypospadias, anterior position of the rectum and incurving of the fourth toe bilaterally. His skin was normal at birth and he was healthy when discharged at 7 days old. At 4 weeks he developed a facial eruption that was initially thought to be impetigo but which failed to respond to several courses of antibiotics. Lesions described as 'paronychiae' were noted on the finger- and toe-nails. While the facial rash persisted and slowly extended, new lesions developed on the arms, legs and perineum.

At the age of 6 months, when referred to our care, he had a symmetrical, non pruritic eruption affecting the face, limbs and perineum with sparing of the trunk. The facial rash was a reticulate, scaly erythema with areas of poikiloderma over the cheeks and chin (Fig. 1). There was focal crusting on the scalp and the margins of the pinnae. The acral lesions were concentrated on the dorsal aspects of the hands, forearms, thighs and knees and the rash was strikingly symmetrical with figurate and annular patterns. Individual lesions were well demarcated, erythematous, hyperkeratotic plaques with raised, brown hyperpigmented margins (Fig. 2). Those on the

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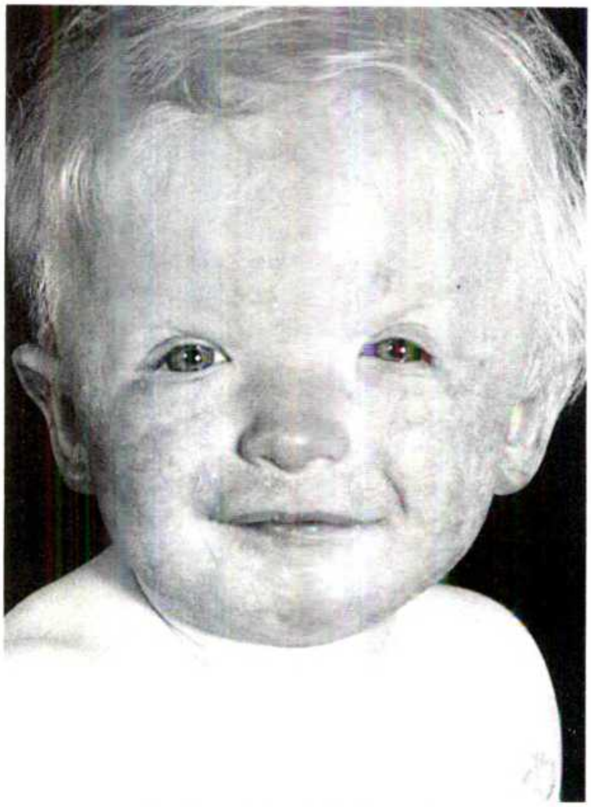

FIGURE 1. Reticulate facial rash.

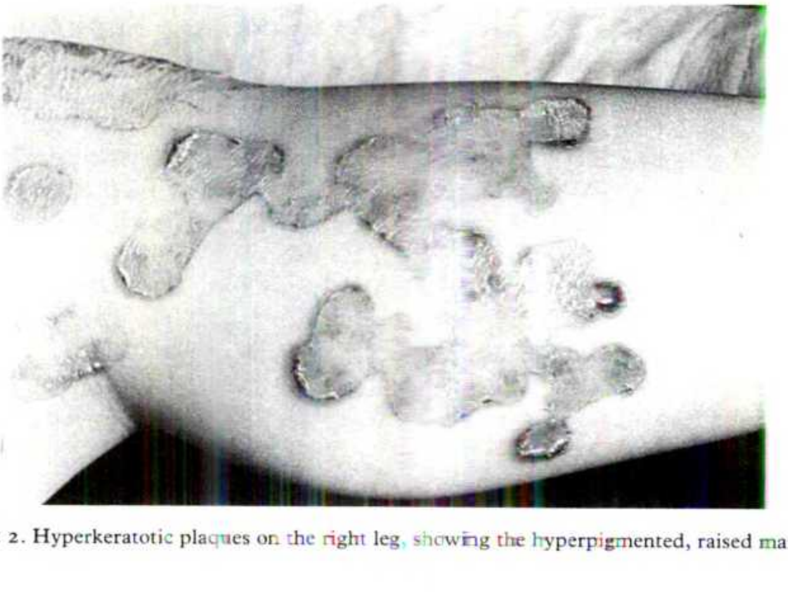

FIGURE 2. Hyperkeratotic plaques on the right leg, showing the hyperpigmented, raised margins.

anterior thighs later showed central clearing. Smaller focal hyperkeratotic, horny lesions were present over the knees, thighs, arms and in skin creases and a few residual 'paronychiae' and interdigital fissures were noted. Other notable features included hypotrichosis of the scalp and eyebrows and delayed primary dentition. The nails were normal. Apart from these abnormalities he was otherwise well. There were no focal neurological signs and his developmental progress was satisfactory.

Investigations

Haematological and biochemical investigations were normal. Immune function studies including immunoglobulin levels, complement assays and detailed neutrophil and lymphocyte function tests failed to show any defect. Microbiological studies were unhelpful. Trace metal analysis revealed normal zinc levels on three separate occasions. The serum magnesium was reduced on one occasion, but not when subsequently tested.

Skin biopsies, from separate areas of involved skin on the right hand, were performed at the ages of 7 and 9 months. Both biopsies showed epidermal changes with parakeratosis, acanthosis and several dyskeratotic cells. There was an increase in the number of mitotic figures.

At the age of 13 months a further skin biopsy, through the edge of a lesion on the anterior thigh, was performed. It revealed, in addition to the previous abnormalities, a cornoid lamella and focal vacuolar degeneration of the basal layer characteristic of porokeratosis (Figs 3 and 4). DNA repair studies on fibroblast cultures were normal.

Management and progress

Prior to referral, the appearance and distribution of the rash were thought to resemble acrodermatitis enteropathica. Despite normal serum zinc levels, treatment was commenced with high dose zinc supplements for 2 months, without any benefit. Biotin supplements for 1 month and topical therapy with a variety of antifungal and antibacterial agents, steroids and emollients were also ineffective.

Following the diagnosis of porokeratosis, a trial of etretinate therapy at a dose of 1 mg kg daily was given but after 2 months there was no improvement and this therapy was discontinued. His treatment at present consists of regular emollients.

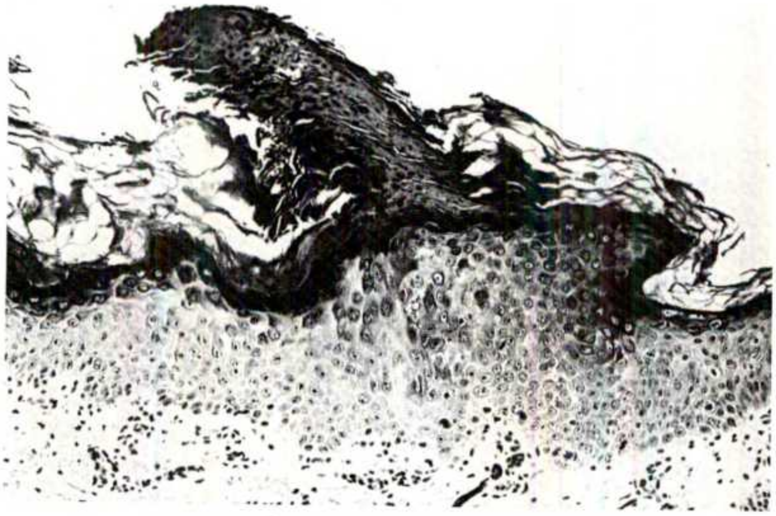

FIGURE 3. Low power view of cornoid lamella.

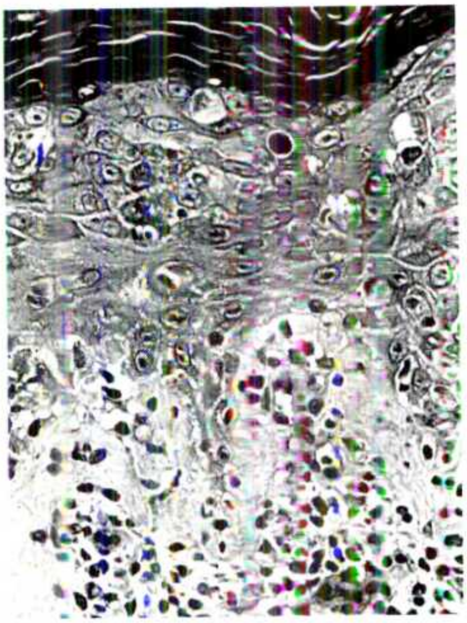

FIGURE 4. High power view showing the epidermal changes.

At the age of 14 months he underwent the first stage of the surgical correction of his craniostenosis. A post-operative wound infection necessitated a course of intravenous antibiotics and this treatment coincided with a lessening of lesional erythema. However, this improvement was transient and occasional new lesions have continued to erupt up to the time of his most recent review. Although some of the skin lesions have shown partial central clearing, the rash has progressed slowly with new discrete lesions arising on the upper neck, flexor aspects of the arms and posterior thighs at the nappy line. The Köebner phenomenon is apparent on the backs of the thighs at the margins of the nappy and also at the site of stitch marks from the most recent skin biopsy.

DISCUSSION

We believe our patient has a genetic disorder of keratinization because of the early onset, symmetrical distribution and the association with other congenital anomalies. The clinical appearance and histological changes suggest an unusual form of porokeratosis.

Gottron's progressive symmetric erythrokeratoderma was considered in the differential diagnosis because of the timing and distribution of the rash, the hyperpigmented raised margin and the Köebner phenomenon. Against this diagnosis were the histological findings of dyskeratosis and the cornoid lamella. The involvement of the skin, hair and teeth raised the possibility of an ectodermal dysplasia. However, sweating was normal, there was no nail dystrophy or palmoplantar keratoderma and the clinical features did not fit with any of the recognized types.[3] The prominent dyskeratosis seen on histology raised the possibility of dyskeratosis congenita but, in the absence of reticulate hyperpigmentation and nail or mucosal lesions and with the early onset, this diagnosis seemed unlikely. Because of the mild poikilodermatous changes on the facial skin, DNA repair studies on skin fibroblasts were carried out. These were normal, excluding a diagnosis of Rothmund–Thomson syndrome. In the light of the findings in the most recent biopsy, we now consider that he has an unusually severe, progressive, symmetrical form of porokeratosis.

Six types of porokeratosis have been described. Classic porokeratosis described by Mibelli in 1893[4] is a rare autosomal dominant disorder of keratinization, more common in males and often commencing in late childhood. Lesions evolve over a number of years into irregular annular plaques with a smooth atrophic centre and a well demarcated, raised, hyperkeratotic border, sometimes showing a central groove. They are usually few in number, asymptomatic, persistent, localized and unilateral, favouring acral sites, thigh and perineum. Lesions may occur on the face and oral mucosa and may also spread to palms and soles. The Köebner phenomenon is a recognized feature and cases of malignant transformation in porokeratotic lesions have been reported.[5] Hyperkeratotic and verrucous variants are recorded[6,7] and some cases have been triggered by immunosuppression.[8]

Four other types of porokeratosis: disseminated superficial actinic porokeratosis, porokeratosis plantaris et palmaris, linear porokeratosis and punctate porokeratosis need not be considered in the differential diagnosis, since clinically they do not in any way resemble this patient's eruption. A sixth variant, reticulated porokeratosis, has been recorded in a single case report.[9] Not included in this classification is porokeratosis striata described by Nekam[10] but now more accurately termed keratosis lichenoides chronica.[11] Although the facial rash in this disorder bears some resemblance to that of our patient the acral lesions differ and the predominant histologic finding is a lichenoid reaction.

These clinically rather diverse conditions are linked by their characteristic histological appearances and by an autosomal dominant mode of inheritance.[12] Their histological hallmark is the cornoid lamella, a slanted column of parakeratotic stratum corneum. The granular layer is absent at the site of the lamella and the keratinocytes beneath are pyknotic with perinuclear oedema. The cornoid lamella is the histological feature which corresponds to the elevated rim seen clinically in a porokeratotic plaque. The centre of the plaque often shows atrophic changes in the epidermis. Liquefaction degeneration of basal keratinocytes and colloid body formation are occasionally seen.

The aetiology of porokeratosis is unknown. It has been suggested[13] that mutant clones of epidermal cells arise and gradually expand outwards. The cornoid lamella at the spreading edge represents the boundary of the expanding clonal population and the normal epithelial cells.

Our patient's eruption most closely resembles the classical or Mibelli variant of porokeratosis. The link between his multiple congenital anomalies and the porokeratosis is unclear. The rarity of each in isolation and the assumption that porokeratosis is dominantly inherited makes a coincidental association unlikely. This raises the possibility that a spontaneous mutation of two or more closely associated genes has occurred or that there is a focal chromosomal defect. The response to treatment is often poor as we found in our patient. There has been a gradual resolution in some lesions and others have become less erythematous but new lesions are continuing to evolve. The outlook for his skin disease is unknown as is the risk of malignant transformation in a porokeratotic lesion.

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Society Proceedings

Proceedings of the 246th Meeting of the Netherlands Society for Dermatology and Venereology, Rotterdam, 3 June 1989

PRESIDENT: PROFESSOR W.A.VAN VLOTEN
HOSTS: PROFESSOR TH.VAN JOOST AND PROFESSOR E.STOLZ

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SUMMARIES OF PAPERS

Micro-organisms, tolerance and autoimmunity

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In 1964 Damian introduced the term 'molecular mimicry' to designate antigens shared by host and parasite.[1] His theory was that antigens shared by host and parasites are not recognized as alien to the host organism and the parasite will be accepted without immune reaction. There is also the possibility that a similarity in antigenic determinants between host and parasite will induce an autoimmune reaction.

Group A streptococci have antigenic determinants in common with myocardial tissue—a fact which might explain the post-infectious myocarditis.[2] *Mycobacterium tuberculosis* and *M. BCG* have antigenic determinants in common with articular cartilage and there are indications that this may play a role in the pathogenesis of rheumatoid arthritis.[3] Myasthenia gravis might be brought about by *Escherichia coli*, *Proteus vulgaris* or *Klebsiella pneumoniae*. These bacteria share antigenic determinants with the nicotine-acetylcholine receptor.[4] *Trypanosome cruzi* shares antigenic determinants both with the myocardium and with nerve tissue. This could explain the autoimmune cardiomyopathy and neuropathy associated with Chagas' disease.[5,6] A study with monoclonal antibodies (MAb) against *M. leprae*, using an indirect immunoperoxidase system with normal human skin as substrate, revealed that 50% of the MAb tested reacted with antigenic determinants in the epidermis. Some of the MAb also reacted with determinants in the corium, e.g. smooth muscle tissue and nerve tissue. The extensive sharing of antigenic determinants between *M. leprae* and the human host might explain the seemingly unbridled multiplication of *M. leprae* in lepromatous leprosy. However, it is quite possible that a *M. leprae* infection, through antigenic mimicry, brings about an autoimmune reaction to the host's own determinants. Such a reaction might explain the granuloma formation and the severe nerve damage in tuberculoid and borderline-tuberculoid leprosy.

Using the Western blot method with an epidermal extract as substrate it was found that normal human serum contains a large number of antibodies which, using this method, react with epidermal antigens. A similar observation was made using normal human serum in an indirect immunofluorescence system with normal human skin as substrate.[7]

Some of the lines seen on Western blot disappear when the serum is adsorbed onto a culturable micro-organism. This might indicate extensive sharing of antigenic determinants between epidermis and micro-organisms.

In view of the above observations it is possible to speculate that a number of the immune dermatoses could be brought about or be maintained by environmental micro-organisms.

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The role of T lymphocytes in the pathogenesis of psoriasis

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The aetiology of psoriasis remains obscure, but there are two main hypotheses as regards its pathogenesis:[1] the epidermal hyperplasia is primary and the dermal inflammatory changes are secondary; the epidermal hyperplasia is secondary. The first hypothesis was supported by studies performed by Van Scott and co-workers.[2] Their results appeared to support the hypothesis that psoriasis is a genetically determined condition inherent to keratinocytes and this resulted in research into the proliferative properties (cell cycle time), the course of cell differentiation (keratin markers) and the aberrant metabolism (cAMP and cGMP contents) of the 'psoriatic' keratinocyte. The findings included the results that keratinocytes from psoriasis lesions produce increased amounts of the mediators of inflammation, particularly leukotriene B_4 (LTB_4) and 12-hydroxyeicosatetraenoic acid (12-HETE), which are also chemotactic factors for myeloid cells and would explain the presence of cells of the non-specific immune system (PMNs, monocytes) in the inflammatory infiltrate.

The second hypothesis postulates that the epidermal lesions in psoriasis are secondary to a primary immunological inflammation in the skin. When monoclonal antibodies (MAb) became available the immunopathology of psoriasis was studied further and it was found that the inflammatory infiltrate in the earliest lesions consisted mainly of $CD4^+$ (inducer/helper) T lymphocytes and monocytes, whereas polymorphonuclear cells were absent. Moreover, T lymphocytes were also present in the epidermis where they were arranged closely to $HLA\text{-}DR^+$ dendritic cells. Such an immunohistological pattern might also be consistent with a type IV allergic reaction. The efficacy of cyclosporin A in psoriasis indicated an involvement of T lymphocytes in maintaining activity and that they play a key role in the pathogenesis of psoriasis.[3–7] T lymphocytes from patients with psoriasis were stimulated to proliferate by autologous epidermal cells. This reaction was described as the mixed epidermal cell–T lymphocyte reaction (MECLR). We have confirmed this reaction and investigated the role of epidermal and peripheral blood antigen-presenting cells in the process. In our *in vitro* system the strength of the autologous MECLR proved to be dependent on the dose of epidermal cells administered. The autologous MECLR proved to be a HLA-DR-restricted process. This reaction was induced, not by peripheral blood antigen-presenting cells but by epidermal cells. In the autologous MECLR the epidermal Langerhans cell did not play the crucial role as observed in allergic contact reactions, as elimination of these cells from the epidermal cell suspension led to merely a slight decrease in the proliferative response. Elimination of the other $HLA\text{-}DR^+$ cells from the epidermal cell suspension led to a virtually complete inhibition of the response. The above results were used to produce a pathogenetic model in which both hypotheses function. $HLA\text{-}DR^+$ dendritic epidermal cells present the 'psoriasis antigen' to T lymphocytes. T lymphocytes become activated in the dermis, proliferate and produce lymphokines (e.g. IL-2 and IFN-γ). In response to the locally produced cytokines, keratinocytes and/or fibroblasts are likewise activated and proliferate. Due to genetically determined factors, these keratinocytes and/or fibroblasts show an increased response to cytokines produced by T lymphocytes. The production of an autocrine growth factor is thereby induced in keratinocytes and/or fibroblasts. Alternatively, there is no negative feedback system for T lymphocyte-induced proliferation in the psoriatic keratinocytes and/or fibroblasts. The two original hypotheses are suggested to be interdependent.

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Yaws in Sumatra, Indonesia, 1988

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Efforts were made to obtain new strains of the yaws pathogen *Treponema pertenue* (*Treponema pallidum* ssp. *pertenue*).

At six health centres situated at a distance from the capital city of Sumatra Barat, Padang, a total of 214 individuals were examined in November 1988 (104 females, 110 males; average age 16 years). Blood samples were obtained from 190 of the patients and TPHA was determined in 21 and VDRL as well as TPHA and FTA-abs in 169 samples (NIPHEH, Bilthoven). Out of the 169 blood samples 89% showed a positive reaction to one or more treponemal tests. Patients were either: 1. Patients with yaws diagnosed clinically and serologically; 2. patients with yaws who had been previously treated; 3. patients with clinical yaws without serological data; 4. doubtful cases: old or new cases of yaws; 5. most probably new cases, all sero-positive; 6. those who had had yaws in the past; 7. sero-positive individuals with a negative yaws history; 8. sero-positive individuals in whom a different diagnosis was made.

Two patients had bone lesions. At the moment yaws is believed to occur in a milder form,[1] however in this study disseminated skin lesions were often revealed. The findings are consistent with reports that yaws is reappearing in many tropical regions.[2]

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Analysis of the type-specific serological response in herpes simplex virus infections

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Herpes simplex virus (HSV) can cause lesions at many different sites. Genital lesions are often (in 80% of cases) caused by HSV type 2, whereas lesions localized elsewhere are usually caused by HSV type 1. However, the nature and location of the lesions often give insufficient information for differentiation between the two types. This requires isolation of the virus. From lesions in an early stage of HSV infection, virus can be isolated without difficulty by means of tissue cultures and typed using specific monoclonal antibodies but from lesions in a late stage virus isolation is often no longer possible. Moreover, HSV infections often take an asymptomatic course. In cases in which virus isolation is no longer possible it would be desirable to establish whether HSV1 or HSV2 infection is involved by means of antibody determination.

Since there is 50% homology between the genomes of the two viruses, the virions have many cross-reacting determinants. This restricts the use of type-specific immunoassays. The demonstration of HSV type-specific antibodies have so far been limited and the most widely used methods are based on demonstrating a difference in neutralizing antibodies against the two types. Such tests are of low sensitivity, particularly if antibodies against both types of HSV are present.

A new method is the Western blot analysis and for this purpose, extracts of cell cultures are infected with HSV1 or HSV2, mounted on a gel. After electrophoresis (SDS-PAGE), the (glyco) proteins are transferred to nitrocellulose (blotting). The blot strips obtained are incubated with patient serum, and any antibody binding is made visible with the aid of stain reactions. Reactions take place both with structural proteins (virion proteins, VP) and with virus-induced proteins (ICP). The band pattern can be further analysed with the aid of densitometry. Most (glyco) proteins show cross-reactions; there are only a few type-specific bands.

The HSV2-specific glycoprotein G2 (92 KDa) can be purified with the aid of affinity chromatography. The purified glycoprotein can be used as antigen in various test systems such as ELISA or dot-blot. The results so far obtained with Western blot analysis and with a dot-blot using purified glycoprotein are promising.

Evaluation of consensus development conference on cutaneous melanoma in The Netherlands

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Marked differences in the recommendations concerning the diagnosis and treatment of cutaneous melanoma in the protocols of various hospitals and medical centres in The Netherlands led to the organization of a consensus meeting on this subject under the auspices of the National Organization for Quality Assurance in Hospitals (CBO) on 15 September 1984. The text of the consensus statement was sent to the 269 participants and published in the Dutch Medical Journal in 1985.

For evaluation of the effect of these guidelines on the daily practice of the specialists in question, pathologists' reports dating from 1983 (1 year before the consensus meeting) and from 1986 and 1988 (2 and 4 years after the meeting, respectively) were analysed. The data were derived from 19 laboratories associated with the Pathological Anatomical National Automated Archive of the Dutch Society of Pathology. The present evaluation is confined to the type of the diagnostic excision by the physician for which in the consensus text the following recommendations were made: 'For the diagnosis of a skin lesion suggesting melanoma, an excision biopsy should in principle be performed. Other types, e.g. the incisional biopsy, should not be considered.'

Of the reports received, 1091 were suitable for analysis, i.e. 324 from 1983, 383 from 1986, and 384 from 1988. Ninety-four of the melanomas had been diagnosed by a general practitioner,

TABLE 1. Occurrence of partial biopsies according to discipline. (Values in parentheses are percentages)

	1983	1986	1988
Dermatologists	22 (45)	29 (39)	12 (18)
General surgeons	9 (4)	5 (3)	3 (1)
Plastic surgeons	0 (0)	0 (0)	0 (0)
General practitioners	2 (9)	4 (10)	1 (3)
Total	33 (10)	38 (10)	16 (4)

630 by a surgeon, 151 by a plastic surgeon, 190 by a dermatologist, and 26 by specialists in other disciplines.

General and plastic surgeons performed partial biopsies only in a small percentage of the cases (1983, 3%; 1986, 2%; 1988, 1%) whereas dermatologists performed many. Far fewer incisional and punch biopsies were performed by dermatologists in 1988 than in 1983 and 1986 (1983, 45%; 1986, 39%; 1988, 18%) (Table 1).

The number of partial biopsies dropped sharply after the consensus meeting. In both 1983 and 1986, 11 dermatologists had performed an incisional biopsy on a suspected case of melanoma; in 1988, there were only two.

The comparison between dermatologists and non-dermatologists has only a limited value because the referral pattern is different.

We conclude that the primary approach of the clinicians, especially the dermatologists, with respect to cutaneous melanoma has improved appreciably after the consensus meeting held in 1984.

CASE REPORTS

Cutis verticis gyrata

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History. A 25-year-old man presented with a swelling in the occipital region which he had since birth. The lesion neither itched nor bled, but was occasionally inflamed. Recently the patient had noticed bald patches developing in the affected region. There was no family history of any skin abnormalities.

Examination. The occipital region showed gyrus-like elevations of soft consistency. The skinfolds did not disappear when traction was applied to the surrounding healthy skin. The skin colour had not changed and there were no signs of inflammation. The growth of the hair was very thin in the affected region, especially in the grooves.

Neurological and ophthalmological examinations showed no abnormalities.

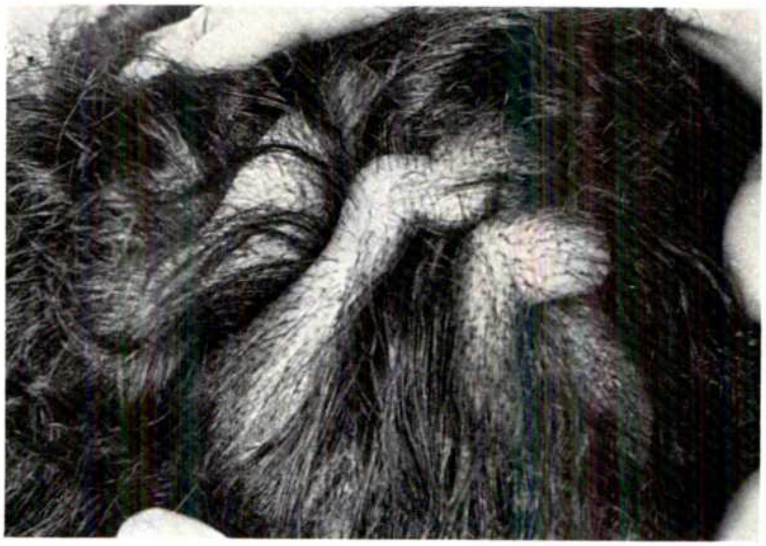

FIGURE 1. Cutis verticis gyrata.

Histology and electron microscopy.The epidermis was orthokeratotic and there was marked thickening of the papillary reticular dermis with cell poor and rich areas. Fairly fibroblast-rich fasciculi were shown extending in the subcutis. Electron microscopy revealed fibroblasts in the cell-rich areas and no naevus cells were seen.

Comment. Cutis verticis gyrata is a morphological description and not a disease entity. The lesion is mainly localized to the hairy scalp, particularly in the occipital region, and comprises skin folds and grooves resembling the gyri of the human cerebrum. The condition is usually asymptomatic but accumulation of material in the grooves may cause complaints about a foetid odour, burning sensation, pruritus or irritation. A primary and a secondary form can be distinguished. The former is often found in association with mental deficiency, epilepsy, cerebral palsy, cranial anomalies (microcephaly), ophthalmological abnormalities (cataract, strabismus) or a combination of these. As in our patient, the condition is rarely encountered in otherwise normal, healthy persons.

The secondary form results from inflammation, trauma or due to a melanocytic naevus. It is also seen in association with neurofibromata, tuberous sclerosis, acromegaly and leukaemia. There is sometimes familial occurence of cutis verticis gyrata based on an autosomal recessive transmission. Treatment consists of surgical excision.

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Dermatitis herpetiformis

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History. A 46-year-old man suddenly developed a generalized bullous dermatosis which was diagnosed elsewhere as dermatitis herpetiformis and treated with dapsone and a gluten- and iodine-free diet. Gastroduodenoscopy at that time revealed normal features and histological examination of a duodenal biopsy specimen showed no specific changes. After a good initial response to the therapy exacerbations occurred. The lower legs and feet were very tender, but the patient never suffered from pruritus.

Examination. The face and the backs of the hands showed a moderately well defined erythema comprising multiple, taut bullae, erosions and crusts; Nikolski's sign was negative. On the lower abdomen and thighs particularly, there were irregular depigmented areas at sites where bullae had been present.

Histology and immunofluorescence studies. There was a subepidermal bulla with fibrin, erythrocytes, segmented granulocytes and nuclear debris. The superficial dermis showed a vasculitis with marked endothelial swelling with a perivascular inflammatory infiltrate and nuclear debris.

Immunofluorescence. There was a fine granular fluorescence along the basement membrane zone of IgA and this was pronounced at the tops of papillae and, to a lesser extent, in the papillary dermis. There were granular deposits of complement at the basement membrane zone, mainly at the tops of papillae.

Treatment. The dose of dapsone was increased from 50 to 100 mg daily and despite temporary

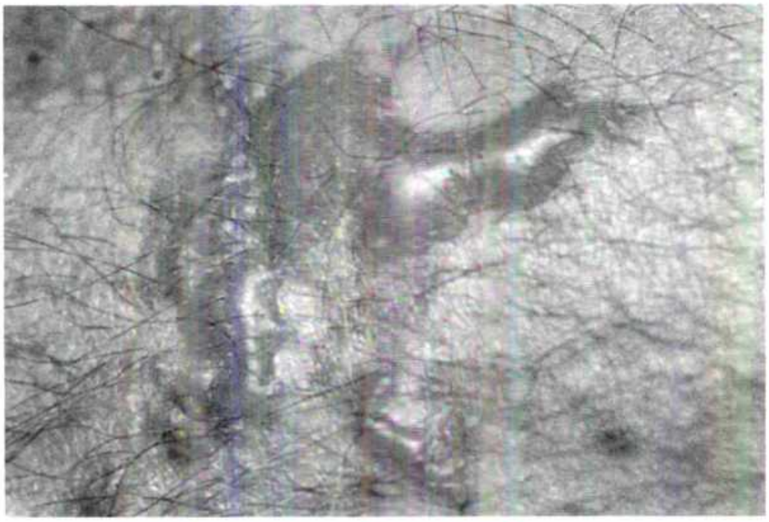

FIGURE 1. Dermatitis herpetiformis with atypical manifestations.

haemolysis there was a rapid improvement. However, the further course was characterized by remissions and exacerbations despite therapy that included a gluten- and iodine-free diet. The dapsone medication was recently supplemented with 500 mg daily of salazosulphapyridine.

Comment. The bullous dermatosis in this patient was diagnosed as dermatitis herpetiformis on the basis of the specific immunopathological findings. However, the clinical features were not consistent with that of classical dermatitis herpetiformis. The almost normal duodenal biopsy specimen and the moderate response to diet and medication suggests another diagnosis. However, in view of the rapid recurrence after discontinuation of therapy and the repeatedly unequivocal immunopathological findings, an unusual presentation of dermatitis herpetiformis seems likely.

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Use of the colposcope ('peniscopy') in subclinical human papilloma virus (HPV) infection of the penis

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History. A 27-year-old patient had for a year been the steady partner of a woman who for 2 years had condylomata acuminata of the vulva. Colposcopy revealed lesions at the level of the cervix which were histopathologically consistent with grade I cervical intraepithelial neoplasia (CIN I). Material taken from the vulval warts had yielded type 11 at HPV hybridization.

The patient himself had not noticed any genital warts. Two years previously he had suffered from an epididymitis of unknown aetiology.

Examination. The external genitals showed no macroscopic lesions. Using the colposcope after application of 5% acetic acid, whitish areas were observed on the inside of the prepuce and around the external urethral orifice (Fig. 1). Within the orifice a small condyloma was visible in the urethra.

Histology. (prepuce) The epidermis showed highly irregular acanthosis and slight parakeratosis. All epidermal layers contained cells showing slight-to-moderate atypia, and at these sites the normal epidermal structure was disrupted. Dyskeratotic cells were also found. The papillary dermis showed a mononuclear cellular infiltrate.

Laboratory tests. HPV typing (*in situ* hybridization) was negative. Urinalysis showed no

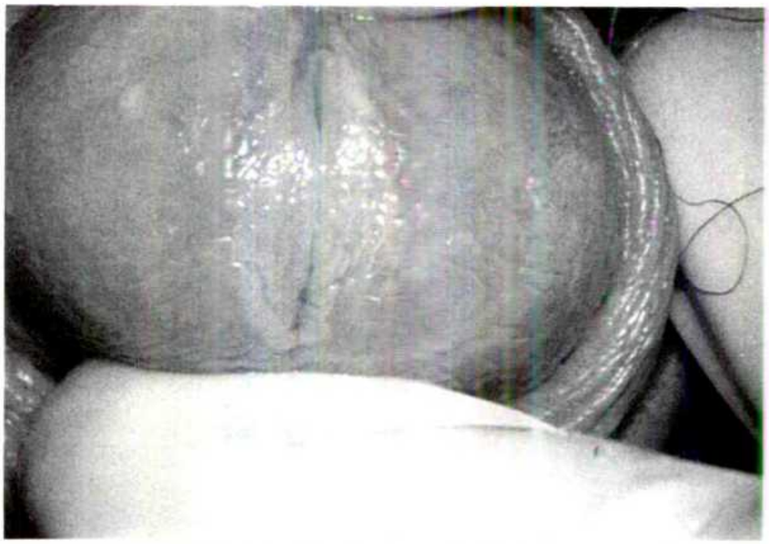

FIGURE 1. Human papilloma virus infection of the glans penis, made visible by colposcopy ('peniscopy') after application of 5% acetic acid.

abnormalities. Syphilis serology was negative. Urethroscopy revealed no lesions apart from a condyloma in the external urethral orifice.

Comment. Data from the literature show that male partners of women with condylomata acuminata and/or CIN lesions also show HPV lesions in 53–59% or even up to 100% of cases. These lesions may be macroscopically visible as flat or raised warts, but subclinical infections also occur in males. With the aid of the colposcope, especially after application of 3–5% acetic acid, these flat subclinical lesions can be detected. In most cases thay are localized on the shaft of the penis proximal to the glans, and on the prepuce. Data from recent studies demonstrate that such subclinical lesions should not be neglected. Their prevalence ranges from 36 to 50%.

As long as examination of partners by colposcopy (especially after application of acetic acid) is not routinely performed, a possible reservoir of virus continues to exist, with potential consequences. The present case illustrates the importance of the use of the colposcope ('peniscope') in these cases.

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Pseudoepitheliomatous, keratotic and micaceous balanitis of Lortat-Jacob and Civatte

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History. A 64-year-old patient was referred to us with persistent abnormalities of the glans penis. In 1974 he had been circumcised for a longstanding phimosis with local fusion of the prepuce. After this operation, painful, itchy crusts and small fissures were reported to have developed on the glans penis. At the same time the external urethral orifice became stenosed necessitating dilatation. Different local treatments produced no improvement.

Examination. Thick white-yellow adherent crusts and scales were seen on the glans penis. The skin had a dry, cracked appearance and some erythematous erosions were present. The general impression was that of an 'armour' surrounding the glans penis.

Histology. (glans penis) The epidermis showed extensive areas of local hyperkeratosis and parakeratosis. The keratinocytes showed large hyperchromatic and polymorphic nuclei with mitoses. The dermis contained an infiltrate of lymphocytes, plasma cells and histiocytes.

Therapy. Patient is currently being treated with 5-fluorouracil cream.

Comment. Pseudoepitheliomatous, keratotic and micaceous balanitis was first described by Lortat-Jacob and Civatte in 1961.[1,2] This skin affection occurs in males who have developed phimosis at a later age. After circumcision the typical clinical features develop. Histopathological

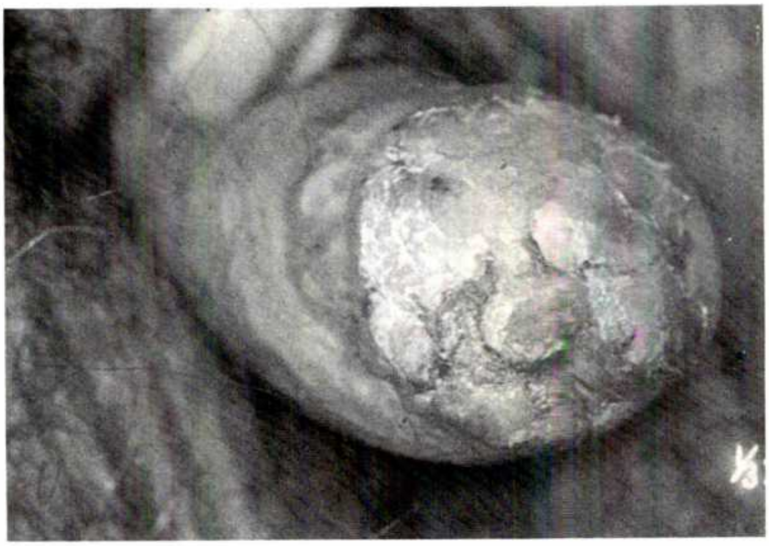

FIGURE 1. Pseudoepitheliomatous, keratotic and micaceous balanitis of Lortat-Jacob and Civatte.

examination reveals pseudoepitheliomatous features. Differentiation from squamous cell carcinoma may pose difficult problems.

This balanitis is regarded as benign, although some investigators believe it to be a verrucous carcinoma, i.e. a low-grade malignancy.[3,4]

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Cutaneous T-cell lymphoma after successful treatment of follicular B-cell lymphoma

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History. In May 1983 a malignant follicular centrocytic-centroblastic non-Hodgkin lymphoma (NHL) was diagnosed in a 46-year-old male patient. Monoclonal IgA-K$^+$ B cells were detected in a resected lymph node. Using chemotherapy with prednisone a complete remission was achieved in December 1983. Regular 6-monthly follow-up revealed no relapses.

Since autumn 1986, sharply circumscribed, superficially scaly red patches appeared and later in the summer of 1988, multiple ulcerating tumours were observed.

Examination. A sharply defined papular erythematous eruption with superficial scaling was present over the entire body surface. Slight lichenification with excoriations were also noted. A generalized dry scaling of the skin was present. The facial skin showed a diffuse erythema with superficial scaling. The lymph nodes were not enlarged.

Histology and immunofluorescence studies. The dermis was diffusely infiltrated by mainly lymphocytic cells. In the epidermis there were small focal collections of mononuclear cells indicating epidermotropism and the formation of Pautrier microabscesses.

Using immunofluorescence most dermal cells were CD3$^+$. A lymph-node biopsy in 1983 showed features consistent with malignant follicular centrocytic-centroblastic non-Hodgkin lymphoma.

Additional investigations. Analysis of T-cell receptor β-chain (TcR-β) genes and also immunoglobulin heavy-chain (IgH) genes was performed. TcR-β gene analysis showed a monoclonal T-cell population in the skin biopsy of 1988, but not in the lymph-node biopsy of

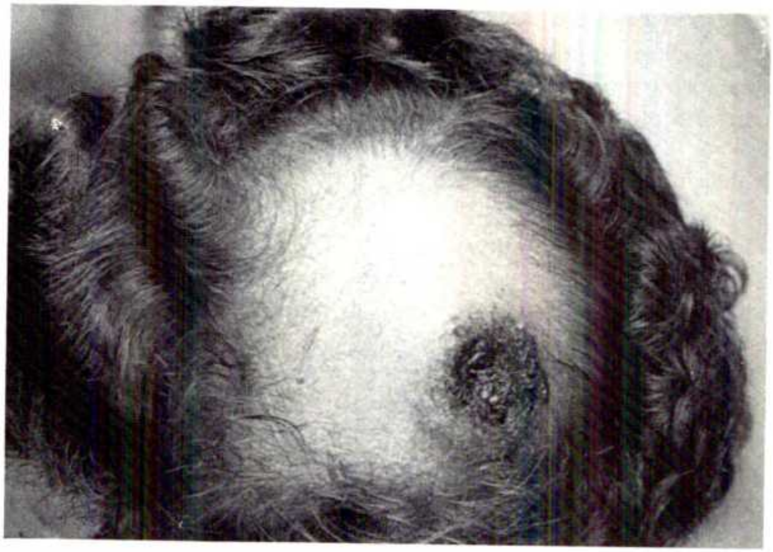

FIGURE 1. Cutaneous T-cell lymphoma after successful treatment of follicular B-cell lymphoma.

1983. IgH gene analysis showed a monoclonal B-cell population in the lymph-node biopsy of 1983 but not in the 1988 skin biopsy.

Comment. The disorder was diagnosed as a cutaneous T-cell lymphoma and developed in a patient who was successfully treated for a follicular B-cell lymphoma. The occurrence of two malignancies in one patient can be explained by a high susceptibility for lymphoma, or the development of a second lymphoma may have been due to previous treatment with cytostatic drugs.

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Diffuse cutaneous mastocytosis

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History. A few hours after birth the patient was referred to the Sophia Children's Hospital in view of extensive skin lesions present since birth. Pregnancy and delivery had been uneventful. The first child in this family was healthy. There was no family history of hereditary or congenital diseases.

Examination. There were multiple plaques scattered over the entire body surface, and some of these had linear vesicles. Almost the entire skin was erythematous, the redness varying in intensity. Several ecchymoses were visible in the scalp. The trunk was covered with a parchment-like membrane. Darier's sign was positive.

Histology. Biopsy 1 (affected skin on leg). There was separation of the epidermis, with the formation of bullae at the dermal–epidermal junction. The dermis contained a band-like infiltrate of cells with ill-defined contours and slight polymorphism and often with oval-shaped nuclei. Biopsy 2 (unaffected skin on leg). The same cellular infiltrate was found in the dermis.

In both specimens, toluidine blue staining revealed many mast cells.

Additional investigations. Direct smear (Tzanck test) of vesicle contents revealed numerous mast cells. The total blood and differential counts as well as the bleeding and clotting times were all normal. Liver and renal function tests were within normal limits. Prostaglandin D2 metabolites in 24-h urine were normal. Histamine-breakdown products in a 24-h urine specimen showed that imidazoles were slightly increased.

Treatment and course. Treatment was started with oxatomide and sodium cromoglycate (Nalcrom®). Bullae and urticarial lesions developed diffusely over the body surface with varying severity. At 1·5 years the bullae stopped appearing and sodium cromoglycate therapy was stopped.

Comment. Mastocytosis can develop at any age, and congenital forms have been previously described.[1–4] The lesions appear before age 2 in 75% of cases. There are only a few reports on diffuse cutaneous mastocytosis with extensive bullae formation in neonates. The skin features are variable and there may be leather-like thickening or smoothness, but it may also show small papules. The colour may range from a diffuse yellow–orange to red. Multiple mastocytomas and extensive bullae may form. In rare cases the skin lesions may be totally absent.

The prognosis of mastocytosis depends on the age of onset. With the onset prior to the age of 5 the prognosis is good and the bullae usually cease by the age of 1–3, and 90% of patients no longer have any symptoms by the time they reach puberty. The involvement of internal organs in young children is rare.

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Netherton's syndrome

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History. After an uncomplicated pregnancy the boy was born as the first child of healthy Turkish consanguineous parents. Immediately after birth a red and scaly skin was noted. By 2 months the child was transferred to the Sophia Children's Hospital (SCH).

Examination. At birth a red skin was noted with peeling on the chin, arms and hands. He did not have the appearance of a collodion baby. One week after birth there was gross lamellar scaling. At the age of 2 months the skin was yellow without any subcutaneous fat. There were fissures but no bullae. The nails were normal and the hair was sparse. There were 'dermogenetic' contractures.

Histology and electron microscopy. The epidermis showed parakeratosis and acanthosis. The granular layer was virtually absent. There was considerable oedema in the epidermis. On electron microscopy desmosomes were irregularly developed and locally absent. There were small numbers of tonofilaments and intercellular oedema (assessment with H.Traupe, Münster, now Nijmegen).

Additional investigations. Hair root examination: 20% anagen, 80% catagen ($n=25$ hairs). Light microscopy of hairs revealed variable hair-shaft diameter but generally the shafts were thin (10–20 μm). Features of trichorrhexis invaginata (bamboo hair) were present. On polarization microscopy there were no special additional abnormalities.

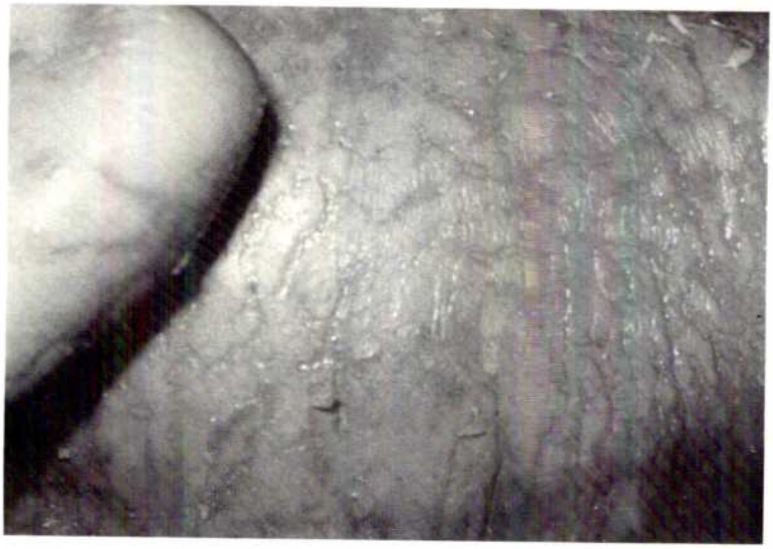

FIGURE 1. Netherton's syndrome.

Course. Recurrent severe infections, a failure to thrive, feeding problems, severe metabolic acidosis, recurrent anaemia, liver failure and dehydration (fluid/protein leakage through the skin) and disturbed thermoregulation were encountered during the course of hospitalization. The child later died.

Comment. Netherton's syndrome is a disorder of keratinization with a non-bullous ichthyosiform erythroderma that often has a fatal outcome within the first few months of life. Typical features are ichthyosiform desquamation with a double collarette (ichthyosis linearis circumflexa), bamboo hair and often an atopic diathesis. It is believed to be an autosomal recessive disorder with considerable penetration in females.

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British Journal of Dermatology (1990) **123,** 271.

Obituary

D.A.Birkett, M.A., M.B., B.Chir., F.R.C.P.

David Birkett died suddenly in brutal circumstances on 3 February 1990. Born in 1934 at Spennymoor, Co. Durham, he went as a Kings Scholar to Durham School, then as a scholar to Emmanuel College, Cambridge, where he obtained a double first class honours degree in Natural Sciences. He then went as a medical student to St Bartholomew's Hospital, London. It was at Bart's under Bob McKenna and Peter Borrie that his interest in dermatology arose. He served for 2 years of National Service as a medical specialist in the Royal Army Medical Corps in Germany and returned to training in dermatology at Bart's and subsequently back to north-east England at the Royal Victoria Infirmary, Newcastle upon Tyne.

His career as a consultant dermatologist was unusual in that he was appointed to Teeside in 1968, moved to Hastings in 1973 and back to Teeside in 1976 where he worked until his death. Here he gave himself an arduous work schedule with eight clinical sessions weekly in five district general hospitals spread over a 20-mile radius.

His main hobby was palaeopathology which he studied and developed to the extent that he became accepted as an international authority on the subject and travelled widely because of it. He was awarded the Medical–Gilliland Travelling Fellowship of the Royal College of Physicians in 1982 and used it to pursue these studies in the U.S.A. and Sweden. True to his retiring nature he could rarely be persuaded to speak in public at medical meetings but his talks on 'old bones' fascinated medical audiences in the region and elsewhere. He rarely missed the afternoon meetings of the dermatology section of the Royal Society of Medicine in London, perhaps not unconnected with the fact that it gave him the preceding morning free to examine more bones in the vaults of the British Museum.

David kept very fit with regular jogging and to prove it, at age 55, completed the London Marathon in 1989 with sponsorship for his Middlesbrough dermatological department.His intellect, impartiality and kind dry sense of humour will be sadly missed by his colleagues who sought and readily obtained his advice in his capacity as Chairman of local medical committees in Teeside. At the time of his death he was Chairman of the Northern Regional Dermatology Sub-Committee and a member of the Committee of the British Association of Dermatologists. He is survived by his children, Simon, Charlotte and Laura.

T.C.H.

PEDIATRIC DERMATOLOGY

Editors

Lawrence M. Solomon *Department of Dermatology, University of Illinois Medical Center*

Nancy B. Esterly *Department of Pediatrics, Children's Hospital of Wisconsin*

Pediatric Dermatology is an international journal which is closely associated with the Society for Pediatric Dermatology and the International Society of Pediatric Dermatology. Its primary purpose is to publish original papers relating to clinical and laboratory investigations, case reports, review articles relevant to the speciality of paediatric dermatology, editorial comments and book reviews.

Pediatric Dermatology aims to further the knowledge of both paediatricians and dermatologists regarding skin diseases of infants, children and adolescents. It particularly supports the contention that skin diseases are ultimately related to the workings of the organism as a whole and may reflect physiological as well as psychological alterations. The journal therefore welcomes contributions which are critical, authoritative and directly related to the structure, function and diseases of the skin. These studies may be biochemical, pharmacological, histological, immunological, electron microscopic, or clinical in nature.

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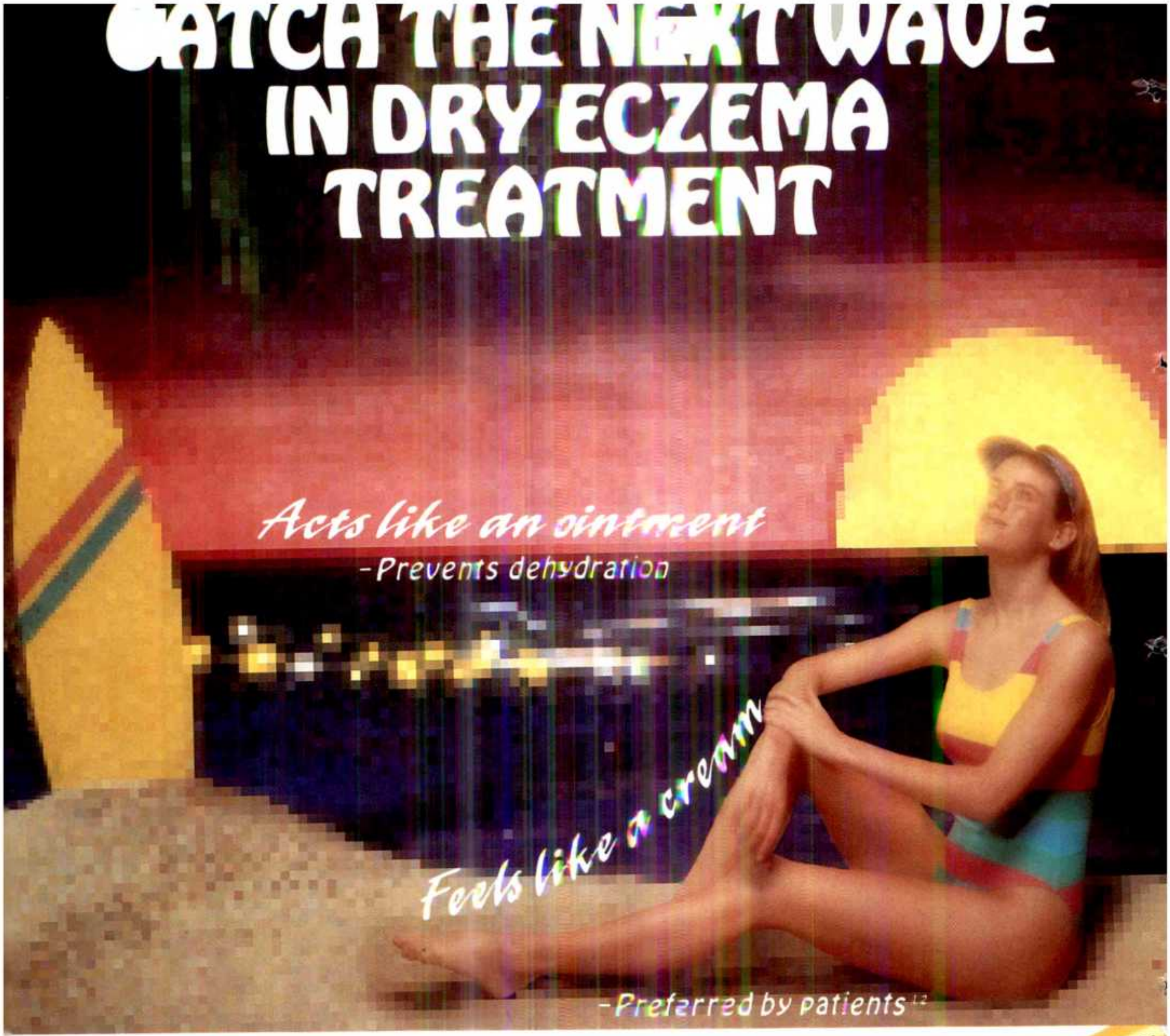

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British Journal of Dermatology (1990) **123**, 273–277.

Correspondence

PUVA therapy of chronic actinic dermatitis: a 5-year follow-up

SIR, In view of your recent article[1] on the treatment of chronic actinic dermatitis with azathioprine, we wish to present a follow-up of our report in this journal on PUVA therapy for this condition.[2] A more detailed review of the cases has appeared elsewhere.[3]

Of our original four cases, all men, who had a mean duration of 8·7 years of light sensitive eczema (range 6–15 years), three have remained symptom free and attend once monthly for PUVA receiving approximately 10 J/cm^2 of UVA each time. One patient has died of cardiovascular disease. Two further patients are undergoing PUVA therapy and remain symptom free.

As stressed in our original article, the main problems arose in the early phase of treatment when the PUVA dosage was very gradually increased by 1-min increments. PUVA was given only twice weekly immediately followed by the application of a topical steroid. We now use hydrocortisone-17-butyrate (Locoid®) applied for 24 h after each PUVA treatment. Fine tuning of PUVA dosage is necessary during the first 3 weeks and all, so far, have in the initial phase been treated as in-patients. We now administer PUVA to light exposed areas only, the patients wearing open-necked short sleeved shirts and pyjamas during irradiation.

The quality of life of these patients is dramatically improved and they can live normally without being protected from the light.

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Fluconazole levels in human epidermis and blister fluid

SIR, In recent years superficial fungal infections have been effectively treated with oral azole antifungal agents. Since the plasma levels of these drugs cannot necessarily be related to their therapeutic effect, we have studied whether fluconazole, a new bis-triazole agent, is distributed in the epidermis following oral administration.

Fluconazole 50 mg daily was given to 12 healthy volunteers for 14 days. Suction blisters were raised after the first and last dose of the drug and after 5 and 10 days following the course of treatment. Blood samples were taken immediately after the first dose and 3 h after the first and last doses and 5 and 10 days later. The concentrations of fluconazole in the blister fluid, in the epidermis of the blister roof and in the plasma were measured by gas chromatography/mass spectrometry.[1] The concentrations of fluconazole in the plasma and blister fluid were similar on all occasions. The concentrations in both fluids on day 14 (about 2·9 μg/ml) were approximately 2·5-fold higher than on day 1. This agrees with a half-life of the drug of 25–30 h. The level of fluconazole found in the epidermis was considerably higher, even on the first day of treatment, with means of 5·55 μg/g on day 1 and 6·45 μg/g on day 14. Detectable levels of fluconazole were still present in the skin 10 days after the end of therapy (Table 1).

TABLE 1. Fluconazole concentrations in plasma, blister fluid and epidermis during and after treatment

Day of trial	Plasma (μg/ml)	Blister fluid (μg/ml)	Epidermis (μg/g)
1 (during treatment)	1·03	1·92	5·55
14 (during treatment)	2·84	2·65	6·45
19 (after treatment)	0·27	0·32	3·55
24 (after treatment)	0·021	0·021	1·91

It is not known where in the epidermis fluconazole is distributed and it may be in the deeper part of the stratum corneum or at the surface. From the known distribution pattern of fluconazole[2] it is likely that the drug is present in sweat. However, the high epidermal concentration found as early as 3 h after the first dose suggest that there is a rapid distribution of the drug to the epidermis from the plasma and tissue fluid.

In conclusion, it appears that fluconazole is well absorbed after oral administration and is rapidly distributed to the subcutaneous tissue and epidermis where it accumulates. A similar distribution and accumulation has been demonstrated for ketoconazole.[3] Preliminary studies have shown that oral fluconazole is effective and safe in the treatment of skin fungal infections.[4]

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Is 'venous stasis' outmoded?

SIR, We read Dr Burton's challenge[1] to defend the term venous stasis and feel that there is a case to be made for its retention.

In 1929, de Takats *et al.*[2] were the first to measure venous oxygen concentration in the lower leg of patients with venous incompetence. They showed decreased oxygen levels. In the same year Blalock[3] reported increased oxygen levels in a similar group of patients. Blalock provided the explanation for this difference: in the first study, the blood was withdrawn from standing subjects, whilst in his own study, they were lying down. The effect of posture on venous oxygen content in patients with venous insufficiency was only confirmed in 1971.[4] In 1985, the effect of posture on blood flow in patients with venous insufficiency was confirmed both by ourselves[5] and independently by Creutzig *et al.*[6] using the non-invasive method of transcutaneous oxymetry. We found that in recumbent patients, skin oxygen tension was greater than in normal subjects (Fig. 1). This finding negates the hypothesis[7] that pericapillary fibrin deposits cause hypoxia responsible for venous ulcers.

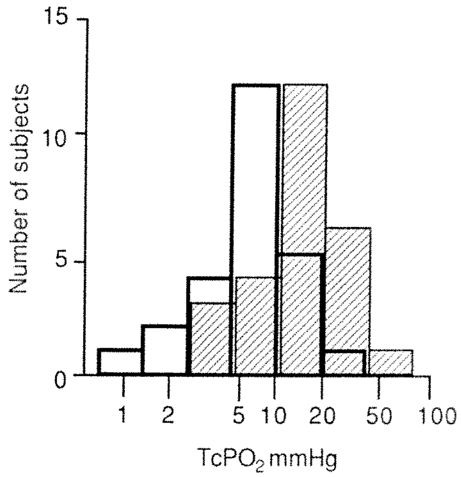

FIGURE 1. Histogram of transcutaneous oxygen tension values in the lower leg 20 cm above the sole of the foot measured in normal subjects and in patients with recently healed venous ulcers. The median value of the observed oxygen tension in the patient group is approximately double the median value in the control group. □, controls, ▨, patients.

We have shown that standing induces hypoxia in both normal subjects and in patients with venous ulcers. This hypoxia is the result of decreased skin blood flow caused by a postural vasoconstrictor reflex elicited by venous distension. It is abolished by exercise in the normal leg. However, in venous incompetence, no rise in oxygen tension is seen with exercise. We therefore postulated[5] that this prolonged hypoxia is responsible for leg ulcers in venous incompetence.

The origin of the word stasis in clinical usage may be traced to the Greek, *στασις*, and was used by the ancient Greek Methodist school to describe dilation of the channels of the body. Butterworth's medical dictionary[8] defines stasis as 'The slowing down or stoppage of flowing of any fluid.' Thus, both the venous distension and the pathologically prolonged hypoxia resulting from the reduction in blood flow which occur on standing in patients with venous insufficiency are described by the ancient and modern usages of the term venous stasis.

We therefore propose that this familiar and useful descriptive term be retained, since direct measurements of skin blood flow using the laser-Doppler flow meter[9] and xenon clearance[10] have confirmed the reduction in skin blood flow on standing demonstrated by oxygen measurement.

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Reply

Sir, I cannot accept Gaylarde and Sarkany's arguments for the following reasons.

They use two different and contradictory definitions of the term stasis. If they insist on retaining the ancient Greek usage of 'dilation of a channel' then I agree that leg ulcers due to venous hypertension are accompanied by venous stasis, since the veins probably are dilated. If they use that definition of the term however, they must also agree that every heart beat produces arterial stasis, and that intradermal injection of vasodilators produces stasis of the skin capillaries. That definition will hardly clarify medical communication, and I hope they can be persuaded to stick to the modern definition—'slowing down or stopping of the flow'.

They have shown that in *recumbent* patients with venous ulcers the skin oxygen content is increased. This lends no support to the idea that there is venous stasis, and there cannot be any significant capillary stasis either, despite Ryan's claims,[1] otherwise the oxygen content would be decreased. Whether pericapillary fibrin deposition causes hypoxia or not is an entirely separate issue, but that hypothesis is certainly not 'negated' by their findings. It could be for example that the increased oxygen pressure is due to the capillary proliferation observed in the skin around leg ulcers, and the oxygen levels might be even higher were it not for the pericapillary fibrin.[2]

They found that the upright *immobile* posture produces a fall in skin oxygen content in normal subjects and in patients with venous hypertension, though the mean oxygen levels remain slightly higher in the ulcerated legs.[3] They attribute this fall to a normal arterial vasoconstrictor reflex, probably secondary to the increased venous pressure produced by standing. This is unlikely to be a major pathogenic factor in the cutaneous changes which follow venous hypertension since it occurs in normal subjects and is not evidence of venous stasis.

We are left with the observation that *exercise* abolishes the cutaneous hypoxia of the leg produced by standing in the normal subject, but not in patients with venous incompetence. Exercise normally causes a fall in venous pressure, which leads to dilatation in the arteriolar and capillary vasculature. It seems likely that the abnormal response seen in patients with venous hypertension is caused by failure of that reflex due to the venous pressure remaining high. Other factors such as pericapillary fibrin deposition could also play a role in maintaining the tissue hypoxia. This is an interesting observation, since it suggests that exercise might not be entirely beneficial for patients with gravitational leg ulcers, but it is not evidence of venous stasis.

In the absence of haemorrhage, blood which reaches the tissues by the arterial system must return by the venous system, and I still maintain that the term 'venous stasis' is entirely inappropriate for a group of patients whose cutaneous blood flow is increased when they are recumbent, and normal when they are standing still, and I can see no evidence that the so-called 'signs of venous stasis' are due to, or even accompanied by, slowing of flow in the veins. Since the term leads to conceptual confusion, let us abandon it.

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Cutaneous reactions database closure

SIR, In July 1988, dermatologists from the U.K. were advised of the establishment of the speciality-based spontaneous reporting centre for the cutaneous side-effect of drugs.[1] This cutaneous reactions database (CRD) sited at the Institute of Dermatology, St Thomas's Hospital was a Committee on Safety of Medicines (CSM) joint collaborative project, funded by the Skin Disease Research Fund for a trial period of 2 years. We are grateful to the 60 dermatologists who did send in a total of 74 reports during the 20 months of its operation.

The group of drugs that had the most adverse reactions reported were the antibiotic group (16), followed by the antirheumatic group (15), drugs for cardiovascular conditions (11), antiepileptic drugs and antimalarials (4). Reactions to three topical agents were reported. The remainder were reactions to individual drugs. In addition to the suspected drug, 47 patients had taken on average two other drugs.

The age of subjects with suspected reactions varied from 18 months to over 80 years, with 66% over 50 years of age. There were 40 females, 33 males, sex unspecified in one report.

The most common type of skin reaction reported was photosensitivity (8) due to drugs that were known photosensitizers such as moduretic, piroxicam, ketoprofen and minocycline. There were seven reports of toxic epidermal necrolysis, three from septrin, one each from piroxicam, mefenamic acid, phenytoin and azathioprine. These were the most serious reactions, and of the four deaths reported three were from this group. The next most common were eczematous reactions (7) two of which were due to contact dermatitis from topical preparations. There were six lichenoid reactions, four of which were due to antimalarials. There were five reports of vasculitis, with one fatality (captopril). There were reports of the known but rare side-effect of pemphigus from penicillamine (one report) and (one report) captopril. There were three reports of hyperpigmentation: two from minocycline (including in one associated ocular hyperpigmentation) and one from captopril. Fixed drug eruptions were reported from a disparate groups of drugs including ranitidine, azathioprine, naproxen and septrin.

An algorithm for assessing causal relationships between drug and event was followed based on previous experience of the suspected drug, possible alternative aetiology of the event, timing of events, patient reaction to withdrawal/re-challenge. Forty-two were thought to be possible drug induced reactions, 24 probable, two unlikely and six not able to be assessed from the information given. The reactions reported are not representative of ones seen in routine dermatological practice but depend on reporter interest, and are biased to more interesting and serious reactions. There was little new information reported excepting increased skin fragility to isotretinoin, and ocular pigmentation from minocycline.

Due to the meagre response indicative more to lack of time and resources than interest, we judge the CRD-CSM insufficiently cost effective and we are terminating the project. The information from the reports which we have received will be retained by the CSM. In accordance with the Data Protection Act we are destroying our computer records.

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REFERENCE

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British Journal of Dermatology (1990) **123**, 278–280.

Book Reviews

Essential Aspects of Atopic Dermatitis. G.Rajka (1989). Berlin: Springer-Verlag. Pp. 261. ISBN 0-387-51165-2. Price £71.00.

Quite simply, this is a superb starting point and reference source for all those with an interest in atopic dermatitis, particularly those planning any research project. The author is Professor Rajka of Oslo, who has spent a large part of his professional life working on atopy. He has gathered together an encyclopaedic collection of references on all aspects of atopic dermatitis.

The text is divided into 12 main sections, the largest being devoted to clinical aspects, three devoted to patho-mechanisms of disease, and one on management. My only mild criticism, which would apply to any current work on atopy, is that at present we do not have a synthesizing theme linking the diverse reported abnormalities in this disease.

The book is a welcome addition to any library. Its personal purchase is highly recommended.

Rona M.Mackie

Pharmacology of the Skin II. Edited by M.W.Greaves and S.Shuster (1989). Berlin: Springer-Verlag. Pp. 587. ISBN 3-540-50277-7. Price: £207.

To the non-specialist, the treatment of skin disease must often seem to reflect the personal preferences of the dermatologist rather than the application of results of controlled clinical trials or sound scientific principles. This stems inevitably from the empiricism of the past, and, although much of this remains, this volume completes a comprehensive review of the pharmacology of the skin, providing considerable ammunition with which to counter unenlightened opinion.

Whereas the first volume of the series covered the pharmacology of the skin systems and autocoids, in normal and inflamed skin, *Pharmacology of the Skin II* concentrates more on clinical pharmacology, including basic investigatory techniques. The well referenced chapters, written by acknowledged experts, are arranged in three sections covering: basic pharmacological investigatory techniques; absorption, metabolism and toxicity of cutaneously active drugs; and, finally, the topical and systemic use of drugs in clinical practice. Each chapter reviews current thoughts and provides extensive and up-to-date references. This combination provides a valuable reference book which should be extremely useful for readers either seeking a synopsis of a subject, or requiring a basis on which to build more in-depth research.

The authors have aimed the book primarily towards pharmacologists, but all dermatologists should profit by studying it. The price is staggering and will surely limit its ownership mainly to libraries, but it should find a well-deserved place, and be much used, in all dermatological and in many medical and scientific libraries. It is to be hoped that the editors will be encouraged to update their major work regularly.

J.N.S.Mitchell

Clinical Dermatopathology; A Text and Colour Atlas. A.Stevens, P.Wheater and J.Lowe (1989). Edinburgh: Churchill Livingstone, Longman Group UK Ltd. Pp. 208. Illustrations 528 with 500 in colour, hardback. ISBN 0-443-02583-5. Price £49.50.

There is no doubt that dermatopathology is rapidly evolving into a distinct speciality. For too long when confronted with problem cases general pathologists have had no alternative but attempt to 'wall paper match' black and white photomicrographs.

This book aims to provide both the histopathologist and dermatologist with clinicopathological correlations of common and important skin disorders. It is divided into 24 short chapters including useful sections on laboratory handling of biopsies, dermatopathological terms and the commonest dermatoses and neoplastic conditions are covered. Many of the clinical cases photographed were biopsied at presentation. The team of authors, two teaching hospital pathologists and a general practitioner, surprisingly does not include a dermatologist. The quality of presentation is very high and the accompanying text concise. However, it only provides the reader with basic facts and the authors do not offer any references. Considering the size of the book quite a wide spectrum of common dermatoses is covered. The authors acknowledge that their book is far from comprehensive but claim that it can be used as a practical day to day diagnostic approach to dermatopathology. In my opinion there are several limitations.

Histology is not always demonstrated, e.g. actinic reticuloid, a condition often misinterpreted as lymphoma, is not illustrated. Perhaps due to a shortage of space, important scanning histology is omitted, e.g. juvenile xanthogranuloma, atypical fibroxanthoma, Spitz naevi and malignant melanoma are not shown. Some text, e.g. epithelioid sarcoma, has no accompanying clinical picture or histology. Throughout the book criteria for differential diagnosis of problem lesions, e.g. dysplastic naevi, melanoma, squamous proliferative lesions or early Kaposi's sarcoma, areas of constant cross referral, are neglected. I would like to have seen areas such as processing artefact, normal regional variation and 'invisible dermatoses' more widely covered. Lastly, a minor frustrating aspect is the number of photomicrographs which appear at a 90° angle.

Nevertheless, the authors are to be congratulated as the book is produced to an exceptionally high level of quality and will be a very useful guide to trainee dermatologists and dermatopathologists. For those experienced dermatologists and histologists faced with a complex diagnostic problem investment in more detailed larger atlases recently available are probably preferable.

R. CERIO

Occupational Skin Diseases. 2nd Edition. R.M.ADAMS (1990). Philadelphia: W.B.Saunders. Pp. 706. ISBN 2926-1.

Professor Adams has excelled himself with this new edition of *Occupational Skin Disease*. Although he is still the principal author, he has enlisted the help and experience of his colleagues from all parts of the world, to add their own specialist and personalized valuable contributions to this volume. Professor Adams is the co-author of many of the chapters. These cover all aspects of occupational skin disease, including physical causes of skin disease, occupational cancer, pigmentary changes due to industrial exposure, occupationally induced connective tissue disorders, as well as detailed chapters on the aetiology and general principles of irritation of the skin, and examination of some of the industrial processes associated with the development of skin disease, and toxicological study of percutaneous absorption of chemicals in the skin. There is of course a section on the development of allergic contact dermatitis, cross sensitization tolerance and desensitization.

Some specific allergens and irritants are detailed, for instance metals, rubber, phototoxic compounds and plants. Investigative techniques are discussed, and the use of the computer in the modern study of occupational skin disease is included. Predisposition is considered in the role of atopy in occupational skin disease.

All these chapters are extensively referenced and the reference system varies according to the authors, so that there is no constant reference system throughout the book. There are many tables and diagrams, which are easy to follow, and large numbers of photographs, all of which are in black and white.

This is a marked advance on the previous volume, issued in 1983, and is the only book available today of its kind. It is therefore invaluable for all departments of dermatology, since it is the definitive work on the development of occupational skin disease, and its relation to dermatology in general and occupational health in general.

The contents have been expanded to include all the modern and new industrial processes, including computer terminal usage, semi-conductor industry, petroleum products, etc., as well as considering the more traditional and better known sources of occupational skin hazards.

The authors must be congratulated on the efficient use of space, which has included such a vast amount of information.

ANGELA FORSYTH

Mycobacterial Skin Diseases. Edited by MARWALI HARAHAP (1989). New Clinical Applications Series: Kluwer Academic Publishers. Pp. 142. Price £30.00.

This is a short multi-author book on diseases caused by mycobacteria. It has chapters on microbiological aspects of mycobacteria and leprosy, tuberculosis of the skin, atypical mycobacterial infections and clinical aspects of leprosy. There are few illustrations and those that are included are in black and white. The main emphasis of the text is on recent advances and practical aspects of the management of these diseases.

While such multi-author works are usually well written, the various authors have often not been given enough space to update the reader adequately on each subject. Unfortunately this book is no exception. The chapter on the pathogenesis of leprosy is a case in point, where there is insufficient space to discuss some of the more exciting advances in the understanding of *M. leprae*, such as antigenic structure and its role in immunomodulation. However, some of the authors have got round this essential problem by producing a brief but well reasoned and readable summary of the field. For instance the section on the mycobacteria and pathogenesis of tuberculosis is an example which is a clear exposition of the ways in which mycobacteria cause disease. The short section on the treatment of cutaneous tuberculosis is equally useful, for a different reason, as it is based on extensive clinical experience not available to most practising dermatologists.

At £30 this book is somewhat expensive for its content. It left this reader wishing that the same team of authors had been asked to contribute to a more substantial book. On the other hand it is well written, easy to read and a useful book to have in the department library until the more comprehensive version is produced.

R. HAY

Book Received

Diagnosis and Treatment of Skin Disorders. B. HALDAR (1989).

British Journal of Dermatology (1990) **123,** 281.

News and Notices

European Society for Dermatological Research
Clinically Orientated Symposium on Bullous Disorders
London, 24–25 January 1991

A clinically orientated symposium on bullous disorders will be held at Regent's College, Regent's Park, London on 24–25 January 1991. The meeting is being organized by Professor Robin Eady and Dr Martin Black of the Institute of Dermatology, St Thomas's Hospital, London, and will be open to members and non-members of the ESDR. The programme will include invited lectures as well as free communications (oral and posters) on clinical and basic scientific aspects of bullous disorders.

Further details including registration and abstract forms are available from Mrs Rosemary Barton, British Association of Dermatologists, 3 St Andrew's Place, London NW1 4LB, tel. 01-487 4475.

Computer Applications in Dermatology
28–29 September 1990, Cardiff

A 2-day symposium organized by the Computer Application Working Group of the British Association of Dermatologists will be held at the University of Wales College of Medicine in Cardiff, 28–29 September 1990. The symposium will consist of invited lectures, free communications and software demonstrations. For further information please contact Dr A.Y.Finlay, Department of Medicine (Dermatology), University of Wales College of Medicine, Heath Park, Cardiff CF4 4XN, U.K.

Introductory Course on the Biology of the Skin, Cambridge, December 1990

The annual introductory course 'The Biology of the Skin' will be held in Fitzwilliam College, Cambridge, 10–14 December 1990. This course is primarily for registrars and postgraduate students at an early stage of their training in dermatology but is open to other interested practitioners. Closing date for applications 23 November 1990. Further information may be obtained from the Conference Secretary (BOS), Postgraduate Medical Centre, Clinical School, Addenbrooke's Hospital, Hills Road, Cambridge CB2 2QQ, U.K. Tel. Cambridge (0223) 212216.

Fourth International Symposium on Atopic Dermatitis
Bergen, 26–30 May 1991

The Fourth International Symposium on Atopic Dermatitis will be held in Bergen on 26–30 May 1991 at the Hotel Norge. The scientific programme will include all aspects of atopic dermatitis. For further information, contact Professor Georg Rajka, Department of Dermatology, Rikshospitalet, 0027 Oslo 1, Norway.

European Society for Pigment Cell Research,
3rd Scientific Meeting, Amsterdam, 8–11 September 1991

The 3rd Scientific Meeting of the ESPCR will be held at the Academic Medical Centre, Amsterdam on 8–11 September 1991. The Organizing Committee is under the chairmanship of Dr Wiete Westerhof of the Department of Dermatology at the Academic Medical Centre. The meeting will provide an interdisciplinary forum for presentations and discussions concerning new biological, biochemical and clinical developments in pigment cell research.

For further information please contact: the Secretariat (3rd ESPCR Scientific Meeting), c/o Bureau PAOG, Tafelbergweg 25, 1105 BC Amsterdam, The Netherlands.

NOTICE TO CONTRIBUTORS

The *British Journal of Dermatology* publishes original articles on all aspects of the normal biology, and of the pathology of the skin. Originally the Journal, founded in 1888, was devoted almost exclusively to the interests of the dermatologist in clinical practice. However, the rapid development, during the past twenty years, of research on the physiology and experimental pathology of the skin has been reflected in the contents of the Journal, which now provides a vehicle for the publication of both experimental and clinical research and serves equally the laboratory worker and the clinician. To bridge the gap between laboratory and clinic the Journal publishes signed editorials reviewing, primarily for the benefit of the clinician, advances in laboratory research, and also reviews of recent advances in such aspects of dermatology as contact dermatitis, therapeutics and drug reactions. Other regular features include book reviews, correspondence and society proceedings. The Journal is the official organ of the British Association of Dermatologists and also of the Netherlands Association for Dermatology and Venereology, but it attracts contributions from all countries in which sound research is carried out, and its circulation is equally international.

Papers accepted become the copyright of the Journal. This Journal is covered by *Current Contents*, *ISI/BIOMED*, *Science Citation Index* and *ASCA*.

Manuscripts. Three copies of all material for publication should be sent to the Editor, Professor S. S. Bleehen, Dermatology Department, Royal Hallamshire Hospital, Sheffield S10 2JF. Manuscripts should be typewritten on one side of the paper only, with a wide margin, be double spaced, and bear the title of the paper, name and address of each author, together with the name of the hospital, laboratory or institution where the work has been carried out. The name and full postal address of the author who will be responsible for reading the proofs should be given on the first page. The author should keep a carbon copy of his manuscript. The Editorial Board reserves the right to make literary corrections.

Abbreviations and units. Full names with uncommon abbreviations must be given with the first mention: new abbreviations should be coined only for unwieldy names and should not be used at all unless the names occur frequently. In the title abbreviations should be identified, in the introduction and discussion they should be used sparingly. Abbreviations of units should conform with those shown below.

gram(s)	g	minute(s)	min	molar	mol/l
kilogram(s)	kg	centimetre(s)	cm	millilitre(s)	ml
milligram(s) (10^{-3}g)	mg	second(s)	s	gravitational acceleration	***g***
microgram(s) (10^{-6}g)	μg	cubic millimetre(s)	mm^3	micrometre(s)	μm
nanogram(s) (10^{-9}g)	ng	millimetre(s)	mm	per cent	%
picogram(s) (10^{-12}g)	pg	millicurie(s)	mCi	isotopic mass number placed as	^{131}I
hours(s)	h	milliequivalent	mmol		

These and other abbreviations and symbols should follow the recommendations of: *Units, Symbols and Abbreviations, A Guide for Biological and Medical Editors and Authors* (1977) The Royal Society of Medicine, London.

Illustrations should be referred to in the text as 'Figs', and be given Arabic numbers, and should be marked on the back with the name(s) of the author(s) and the title of the paper. Where there is any possible doubt as to the orientation of an illustration, the top should be marked with an arrow. Each figure should bear a reference number corresponding to a similar number in the text. Photographs and photomicrographs should be *unmounted* glossy prints and should not be retouched. Colour illustrations will be accepted when found necessary by the Editor, although the author will be expected to bear the cost. Diagrams should be on separate sheets; they should be drawn with black ink on white paper or feint-ruled graph paper and should be about twice the size of the final reproduction. Lines should be of sufficient thickness to stand reduction. Each illustration should be accompanied by a legend clearly describing it; these should be grouped on a separate sheet of paper.

There should be as few tables as possible and these should include only essential data; they should be typewritten on separate sheets and should be given Arabic numbers.

References. Only papers closely related to the author's work should be referred to; exhaustive lists should be avoided. References should be given in the Vancouver style, i.e. references appear as superscript numbers in the text, e.g. '. . . our previous report[1] and that of Smith *et al.*[2] . . . ', and are brought together numerically in the reference list at the end of the article in order of their first mention in the text. References to articles and papers should mention: (1) name(s) followed by the initials of the author(s), up to four authors: if more than four authors, include first three authors followed by *et al.*; (2) title of paper; (3) title of journal abbreviated in the standard manner; (4) year of publication; (5) volume; (6) page numbers of the article. *Thus*: Cunliffe WJ, Shuster S. The rate of sebum excretion in man. *Br J Dermatol* 1969; **81:** 697–9. References to books and monographs should include: (1) author(s) or editors(s); (2) paper (if necessary) and book titles; (3) edition, volume, etc.; (4) place; (5) publisher; (6) year; (7) page(s) referred to. *Thus*: Beare JM, Wilson Jones E. Necrobiotic disorders. In: *Textbook of Dermatology* (Rook AJ, Wilkinson DS, Ebling FJ, eds), 2nd edn., Vol. 2. Oxford: Blackwell Scientific Publications, 1972; 1066.

Page proofs will be submitted without the original typescript to the author for proof-correction (see under 'manuscript') and should be returned to Blackwell Scientific Publications within three days. Major alterations from the text cannot be accepted.

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